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# EFFECT OF LEAF RUST (PUCCINIA TRITICINA) ON YIELD, PHYSICAL CHARACTERS, AND COMPOSITION OF WINTER WHEATS[1]

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## INTRODUCTION

There is little published information regarding the effect of the various cereal rusts on the composition of the host plants and grain, although several studies on the effect of rust on yield have been published. At La Fayette, Ind., the season of 1931 was extremely favorable for a study of the effect of leaf rust of wheat (*Puccinia triticina* Eriks.) on the composition of plants and grain and on the yield of grain and straw. A severe leaf-rust epiphytotic developed on susceptible varieties soon after the flowering period. Other diseases either were entirely absent or were present in an unimportant degree. The season was favorable for large yields. The plants developed a strong growth of straw that withstood severe winds without lodging, a particularly fortunate condition for studying the effect of rusts on cereals. A similarly fortunate combination of circumstances may not reoccur soon, which is the justification for presenting the data for a single year. A preliminary report of this work has been published by the writers (*4*).[2]

## METHODS AND MATERIALS

In the fall of 1930, seven varieties of winter wheat, varying in relative susceptibility to leaf rust from extremely susceptible to highly resistant, were sown in 2 series with 4 replications of each variety in each series. The varieties were Fulhard (C.I.[3] no. 8259), Gladden, Kawvale (C.I. no. 8180), Nittany (Pa. no. 44), Purkoff, Shepherd (C.I. no. 6163), and Fultz selection (C.I. no. 11512), a highly leaf-rust-resistant selection from a segregating strain of Fultz. Beginning the following April, one series was dusted with sulphur in order to control leaf rust, whereas the other was not dusted.

The seed was sown by hand at the rate of 6 pecks per acre, this rate being commonly used in Indiana farm practice. Care was exercised to secure uniform distribution in the drill row. The two series were sown on a uniform area separated by three rows of wheat

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[2] Reference is made by number (italic) to Literature Cited, p. 1071.

[3] C.I. denotes accession number of the Division of Cereal Crops and Diseases (formerly Office of Cereal Investigations).

to guard against the drifting of sulphur from the dusted to the nondusted series. Each replication or plot of a given variety consisted of four 18-foot rows 1 foot apart, the 2 outermost serving as guard rows between the varieties. The plots were continuous, i.e., separated by only 1 foot, as were the rows within the plots. The four replicated plots of each variety in both dusted and nondusted series were in linear arrangement and extended the entire length of the experimental area.

The grouping of the dusted and nondusted plots in separate series was to prevent the sulphur from drifting to the nondusted plots. This plan made impossible the more desirable systematic distribution of plots commonly in use in comparative yield trials. However, because of the uniformity of the soil and the small land area involved, this system apparently has given satisfactory data from a practical point of view.

A 2–12–6 fertilizer was applied with a disk drill at the rate of 200 pounds per acre prior to seeding. The drill was drawn parallel to the short dimension of the area, whereas the seed was drilled in the opposite direction, to avoid, so far as possible, the influence of any irregularity in the distribution of the fertilizer.

A sulphur dust containing the oxidizing agent, potassium permanganate, was used throughout the experiment. The dust was applied with a bellows-type hand duster at the rate of 100 pounds per acre, the rate being slightly increased in the later dustings. Because there is a rather large wastage of dust in using a bellows-type duster and in treating rows spaced 1 foot apart, heavy applications were necessary. The control of leaf rust was very satisfactory, the percentage of infection reaching only 10 to 15 percent on the dusted plots of very susceptible varieties, in contrast to 100 percent, shortly after flowering time, on the nondusted plots.

In this study the sulphur-dusted plots have been considered as controls, to be used as a basis from which to measure the effect of the rust on the rusted (nondusted) plots because the interest is primarily in the effect of the rust rather than that of the fungicide. It is recognized that the sulphur dust may have slightly altered the normal development of the control plants. The control plots were dusted 11 times during the period of development from the jointing stage to maturity. The dust was applied whenever it seemed necessary because of removal by rains.

In addition to the above-described sowing, a very leaf-rust-susceptible selection of Michigan Amber wheat was sown in the same block in 8 plots of 16 rows each. One extra row between plots and the outermost unharvested rows of each plot served to guard against sulphur drifting. By variations in the time at which the dusting was started in the several plots, four different degrees of severity of rust were produced. These plots were in duplicate for each of the dusting treatments. The plots dusted, beginning at the jointing stage, booting stage, and flowering stage, and the nondusted plots developed 15, 52, 100, and 100 percent, respectively, of leaf rust at maturity. Although no difference could be seen in the amount of rust at maturity on the plants of the last two treatments mentioned, the rust developed to its maximum later on the plants dusted at flowering time, and the foliage remained alive longer than on the nondusted plants.

Notes on leaf rust were taken for each variety for each treatment on June 16, 20, and 27 and July 1. On the first date little killing of the upper leaves had occurred as a result of ripening or rust attack. By June 27 most of the leaves were dead or entirely chlorotic. The average number of living leaves per tiller and the leaf-rust severity for each living leaf per tiller were estimated for a number of representative tillers on these different dates. Rust severity was estimated according to the scale adopted by the Division of Cereal Crops and Diseases. The percentages of rust for June 16 represent the average estimated amount of rust per tiller on leaves living at that time. The percentages of rust infection recorded for June 20 and 27 and July 1 are based on the maximum amount of rust that had developed prior to any one of the given dates on all of the leaves per tiller that were alive on June 16, even though some or all of them may have been dead when the notes were taken on the later dates.

Yield data were taken from the 2 center rows of the 4-row plots and from 12 center rows of the 16-row plots. The ends of the rows for a distance of 1 foot were discarded in harvesting the plots in order to avoid border effect. In presenting the yield data the probable errors of the means of the replicate 4-row plots have been calculated by the deviation-of-the-mean method (*6*).

Detailed methods pertaining to separate phases of the study are given later.

## EFFECT OF LEAF RUST ON YIELD

### GRAIN

Leaf rust was responsible for very marked decreases in yield of winter wheats, as determined by the comparison of replicated control (sulphur-dusted) plots and heavily rusted plots. The pertinent data are given in tables 1 and 2. The yields of the susceptible varieties, Shepherd, Michigan Amber, and Gladden, were reduced 28.4, 20.8, and 14.8 percent, respectively. The reduction for Nittany, moderately susceptible, was 8.2 percent and for the slightly more resistant Purkoff only 2 percent.

The control (sulphur-dusted) plots of the more resistant varieties, Kawvale and Fultz selection, yielded less than the rusted (nondusted) plots (table 1).[4] The differences amounted to 3.4 and 12 percent, respectively. The difference for Kawvale is not statistically significant, but that for Fultz selection is 3.3 times its probable error. Likewise, the yield of the rusted plots of Fulhard slightly exceeded that of the control plots, although the former were very severely rusted and dusting might have been expected to increase the yield. The lower yields of the control plots of these three varieties suggest the possibility of an inhibitory action of the sulphur on the plant development and that the effect may vary with different varieties.

The reduction in yield of Michigan Amber was closely proportional to the estimated leaf-rust severity (table 2 and fig. 1). Thus, the plots having infections of 15, 52, and 100 percent of late development and 100 percent of earlier development produced average yields of 38.4, 34.2, 31.3, and 30.4 bushels per acre, respectively.

[4] The nondusted plots of Fultz selection, in conformity with the nondusted plots of other varieties, are designated in the tables as rusted plots, although little rust developed on them because of their natural resistance.

TABLE 1.—*Effect of leaf rust on yield per acre of grain, protein, and straw, test weight per bushel, and weight per 1,000 kernels of winter-wheat varieties of differing degrees of susceptibility to leaf rust*

| Variety | C.I. no. | Kind of plot | Maximum leaf-rust severity | Acre yield of grain [a] | Grain loss or gain in rusted plots | Test weight per bushel | Weight [b] per 1,000 kernels | Loss or gain in weight per 1,000 kernels in rusted plots | Acre yield of protein | Protein loss or gain in rusted plots | Acre yield of straw [c] | Straw loss or gain in rusted plots |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Percent* | *Bushels* | *Percent* | *Pounds* | *Grams* | *Percent* | *Pounds* | *Percent* | *Pounds* | *Percent* |
| Shepherd | 6163 | Rusted | 100 | 29.2±0.71 | −28.4 | 58.5 | 30.9 | −7.5 | 156.7 | −38.3 | 4,852±142 | −13.3 |
| | | Control | 13 | 40.8± .99 | | 59.6 | 33.4 | | 254.1 | | 5,595±163 | |
| Gladden | | Rusted | 100 | 37.3± .91 | −14.8 | 57.6 | 31.3 | −12.1 | 185.0 | −25.6 | 4,811±141 | −14.2 |
| | | Control | 15 | 43.8±1.06 | | 59.1 | 35.6 | | 248.6 | | 5,606±164 | |
| Fulhard | 8259 | Rusted | 100 | 35.1± .84 | +1.2 | 62.2 | 30.9 | −5.2 | 188.5 | −14.6 | 4,762±139 | −7.6 |
| | | Control | 10 | 34.7± .85 | | 62.9 | 32.6 | | 220.6 | | 5,152±150 | |
| Nittany | | Rusted | 75 | 34.5± .84 | −8.2 | 58.1 | 41.4 | −3.0 | 194.4 | −15.5 | 5,602±164 | −10.7 |
| | | Control | 10 | 37.6± .91 | | 58.7 | 42.7 | | 230.0 | | 6,274±183 | |
| Purkoff | | Rusted | 65 | 39.5± .96 | −2.0 | 58.6 | 31.2 | −4.9 | 212.5 | −11.8 | 5,467±160 | +.3 |
| | | Control | 8 | 40.3± .98 | | 59.2 | 32.8 | | 240.8 | | 5,452±159 | |
| Kawvale | 8180 | Rusted | 45 | 42.2± .99 | +3.4 | 60.6 | 32.8 | −3.2 | 228.1 | −0.6 | 4,950±145 | −.6 |
| | | Control | 5 | 40.8±1.03 | | 60.9 | 33.9 | | 229.5 | | 4,980±145 | |
| Fultz selection | 11512 | Rusted | 7 | 43.0± .93 | +12.0 | 59.2 | 32.0 | −1.5 | 245.8 | +10.3 | 5,872±160 | +7.3 |
| | | Control | 1 | 38.4±1.05 | | 58.8 | 32.4 | | 222.8 | | 5,472±172 | |

[a] Average of 4 replicated plots.
[b] Average, on air-dry basis, of 4 replicated plots of Shepherd, Nittany, and Fultz selection; determined on composite samples of threshed grain from 4 replicated plots of Gladden, Fulhard, Purkoff, and Kawvale.
[c] Average of 4 replicated plots on air-dry basis at threshing time.

TABLE 2.—*Effect of leaf rust in different degrees of severity on yield of grain and protein per acre, test weight per bushel, and weight per 1,000 kernels of Michigan Amber wheat* [a]

| Stage of plant maturity at beginning of dusting | Maximum leaf-rust severity | Acre yield of grain | Grain loss in rusted plots [b] | Test weight per bushel | Weight per 1,000 kernels [c] | Loss in weight per 1,000 kernels in rusted plots [d] | Acre yield of protein [d] | Protein loss in rusted plots [b] |
|---|---|---|---|---|---|---|---|---|
| | *Percent* | *Bushels* | *Percent* | *Pounds* | *Grams* | *Percent* | *Pounds* | *Percent* |
| Nondusted | 100 | 30.4 | 20.8 | 58.2 | 32.3 | 8.24 | 163.3 | 30.2 |
| Flowering | 100 | 31.3 | 18.5 | 58.5 | 32.9 | 6.53 | 173.9 | 25.6 |
| Booting | 52 | 34.2 | 10.9 | 59.3 | 34.4 | 2.27 | 197.8 | 15.4 |
| Control (jointing) | 15 | 38.4 | ..... | 59.7 | 35.2 | ..... | 233.8 | ..... |

[a] Averages of determinations from duplicate 12 rod-row plots.
[b] Percentages calculated on basis of the plots in which dusting was begun at the jointing stage.
[c] Determined on air-dry basis.
[d] Determined on dry-weight basis.

## STRAW

When the grain was threshed the total weights of the harvested bundles, including grain, were recorded. At that time the bundles had dried in the field for 8 days under excellent drying conditions and were apparently air-dry. The recorded yields of straw (table 1) are average weights for four replicated plots based on these bundle weights minus the weight of the grain threshed from them. The probable errors were calculated in the same manner as were those for the grain yields.

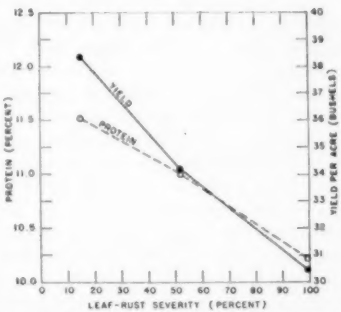


FIGURE 1.—Relation of leaf-rust severity to yield and protein content of Michigan Amber wheat.

The effect of leaf rust on yield of straw of each of the several varieties in the experiment was very similar to that on the yield of grain. In the susceptible varieties, Shepherd and Gladden, the yield of straw was reduced 13.3 and 14.2 percent, respectively, and in the less susceptible Nittany, 10.7 percent. In the moderately resistant varieties, Kawvale and Purkoff, no appreciable difference was noted between the yields of straw from the rusted and the control plots. The rusted plots of Fultz selection, the highly resistant variety, yielded 7.3 percent more straw than did the control plots.

The straw yields of the replicated plots varied considerably and thus led to a much larger probable error in relation to yield than was found for the grain yields. From a statistical standpoint the effect of rust on the straw yields is of doubtful significance. The effect, however, is in harmony with that for the yields of grain.

## NATURE OF YIELD REDUCTION CAUSED BY LEAF RUST

The effect of leaf rust on the number of kernels and weight of grain per head was studied in the following three varieties of winter wheat: Shepherd (susceptible), Nittany (moderately susceptible), and Fultz selection (highly resistant). In addition, the effect of varying severities of leaf-rust infection on kernel number and weight was studied in Michigan Amber (highly susceptible). The variations in the dusting

procedure to produce these varying severities of rust are described above. The pertinent data for all varieties are given in table 3.

### REDUCTION OF NUMBER OF KERNELS PER HEAD

The most heavily rusted plants of the highly susceptible Michigan Amber suffered an average loss of 4.65 kernels per head as compared with the control plants, or a reduction of 16.69 percent. The average numerical kernel loss per head in Shepherd was 4.91 kernels, but, owing to the larger heads, the percentage loss was 13.85 percent, or less than in Michigan Amber (table 3). These losses appear to be statistically significant. In the less susceptible Nittany the loss was only 1.13 kernels per head, or 3.91 percent. The loss was statistically significant, however.

In the very resistant Fultz selection the difference in number of kernels was in favor of the rusted plants, as it was in yield, but this difference was small and not significant. In Michigan Amber the percentage reduction in number of kernels per head was found to vary regularly in proportion to the rust severity.

### REDUCTION IN WEIGHT OF KERNELS

Leaf rust also markedly reduced the weight of kernels in the susceptible varieties. Thus, the weights of the individual kernels from rusted plants of Shepherd and Michigan Amber were reduced on an average of 5.95 and 7.10 percent, respectively, as compared with kernels from the control plants. The loss in weight per kernel of Nittany was only 1.53 percent, whereas that of the rusted plants of the resistant Fultz selection again, as in number of kernels, very slightly exceeded that of the control plants, the kernels being 0.92 percent heavier than those of the controls. Reductions in kernel weight also were proportional to rust severity, as is shown in the Michigan Amber series, although the most important effect of rust on yield was expressed in a numerical reduction of kernels per head. The reduction in weight was not caused by a visible shrinkage of the kernels, for kernels from rusted plants apparently were as plump as those from the control plants.

Mention should be made here of the comparison between weight per kernel presented in table 3, as determined from the 100-head samples of the larger heads of uniform size taken from the standing grain, and the weight per kernel as determined from 1,000 kernels of the threshed grain (tables 1 and 2). The weight of grain in grams per 1,000 kernels as given in tables 1 and 2 may be compared directly with the data in table 3. As would be expected, in every case the weight per kernel of the selected heads is greater than that of the threshed grain. On either basis, however, the same trends may be noted in the effect of rust on kernel weight.

A similar comparison may be made between the reduction in yield per acre of threshed grain (tables 1 and 2) and the reduction in weight of grain per head calculated from the samples of 100 heads (table 3). In Michigan Amber the agreement in percentage losses determined by the two methods is very close, while in Shepherd and Nittany there are considerable differences. Since the data from the threshed grain of the entire plot must be considered the more reliable, the results from the relatively small lots of 100 heads should be accepted as indicative of trends rather than as representative of the actual conditions within the plots.

TABLE 3.—*Effect of leaf rust on kernel formation in winter wheats* [a]

| Variety | C.I. no. | Kind of plot | Maximum leaf-rust severity | Kernels per head | | Weight per kernel | | Total weight of kernels [b] per head | | Proportion of total yield loss caused by— | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean | Loss or gain in rusted plots | Mean | Loss or gain in rusted plots | Mean | Loss or gain in rusted plots | Reduction in kernel number | Reduction in kernel size |
| | | | *Percent* | *Number* | *Percent* | *Mg* | *Percent* | *Mg* | *Percent* | *Percent* | *Percent* |
| Shepherd | 6163 | Rusted | 100 | 30.53±0.31 | −13.85 | 32.56 | −5.95 | 994±9 | −18.99 | 72.96 | 27.04 |
| | | Control | 13 | 35.44± .33 | | 34.62 | | 1,227±11 | | | |
| Nittany | | Rusted | 75 | 27.77± .22 | −3.91 | 43.82 | −1.53 | 1,217±9 | −5.37 | 72.88 | 27.12 |
| | | Control | 10 | 28.90± .24 | | 44.50 | | 1,286±11 | | | |
| Fultz selection | 11512 | Rusted | 7 | 31.42± .36 | +2.45 | 32.97 | +.92 | 1,036±11 | +3.39 | 72.76 | 27.24 |
| | | Control | 1 | 30.67± .26 | | 32.67 | | 1,002±8 | | | |
| Michigan Amber | | Nondusted | 100 | 23.20± .18 | −16.69 | 33.62 | −7.10 | 780±6 | −22.61 | 73.82 | 26.18 |
| | | Dusted in flowering stage | 100 | 24.33± .19 | −12.64 | 33.91 | −6.30 | 825±7 | −18.15 | 69.63 | 30.37 |
| | | Dusted in booting stage | 52 | 25.48± .20 | −8.50 | 35.28 | −2.51 | 899±6 | −10.81 | 78.71 | 21.29 |
| | | Control (dusted in jointing stage) | 10 | 27.85± .22 | | 36.19 | | 1,008±8 | | | |

[a] Determined on composite samples of 100 uniform, ripe heads selected partly from each of 4 replicated plots of Shepherd, Nittany, and Fultz selection and from duplicate plots of Michigan Amber.

[b] Determined on air-dry basis.

### RELATIVE IMPORTANCE OF REDUCTION IN WEIGHT AND NUMBER OF KERNELS

The loss in yield of grain per head caused by leaf rust through reduction in number of kernels has been calculated by multiplying the mean weight per kernel of the grain from the control plants by the mean loss of kernels per head of the rusted plants. The loss through reduction in size of kernel has been calculated by multiplying the mean loss in weight per kernel of the rusted plants by the mean number of kernels per head of the rusted plants. The sum of these losses (table 3) equals the mean total loss per head.

In all cases where leaf rust caused a loss, the reduction in number of kernels accounted for approximately three-fourths of the loss per head and the loss in kernel weight the remainder (table 3). This approximate ratio changed but little, regardless of the severity of the infection or the susceptibility of the varieties. Thus, in Shepherd and Nittany, which varied greatly in the severity of rust, the ratios between the two loss factors were nearly identical. In Michigan Amber the ratios varied somewhat in the plots under different rust attacks, but no significant trends in relation to rust severity are evident.

Since leaf rust was very light during the seedling stage of the grain, it appears unlikely that the number of tillers or heads per plant could have been appreciably affected. This factor has, therefore, been disregarded in considering the manner in which leaf rust reduced grain yields for this year.

The findings for the susceptible varieties were very similar to those of Mains (*14*) and Johnston (*10*), who showed that leaf rust of wheat reduced the yield of grain of infected plants primarily by reducing the number of kernels per head and to a lesser degree by reducing the weight of the kernels.

### EFFECT OF LEAF RUST ON PHYSICAL CHARACTERS OF GRAIN

The most marked visible effect of rust on the grain was a reduction in the proportion of vitreous kernels in the grain from rusted plants as compared with that from the control plants. This physical condition was correlated with a reduced protein percentage content of the grain as shown in tables 4 and 5. All the susceptible varieties studied were thus affected, and in all varieties the difference in kernel color made it possible to distinguish easily the grain of the rusted plants from that of the controls. In the soft wheats the kernels from rusted plants were soft and starchy throughout, whereas those from the control plants tended to have vitreous tips. In the hard and vitreous Fulhard and the semihard Kawvale a physical separation of the kernels could be made into the three classes, (1) soft or starchy, (2) piebald—those comprising mixed starchy and vitreous areas—and (3) vitreous (table 4 and fig. 2). In Fulhard 73.9 percent by weight of the kernels were vitreous and 26.1 percent were piebald for the control plants. Under severe rust attack the ratios were nearly reversed, only 20.4 percent of the kernels being vitreous and 79.6 percent piebald. The same tendency was expressed in Kawvale. This induced starchiness or piebaldness in all varieties is identical with the condition classified as yellow berry in the hard-wheat grain trade. Mains (*14*) has reported that leaf rust tends to increase the proportion of yellow-berry kernels of plants artificially inoculated with leaf rust in the greenhouse.

In Fultz selection, the resistant variety, where only 7 percent of rust occurred on the nondusted (rusted) plots, there were no visible differences between the physical characters of the grain from the control and the nondusted plants (table 5). This was correlated with

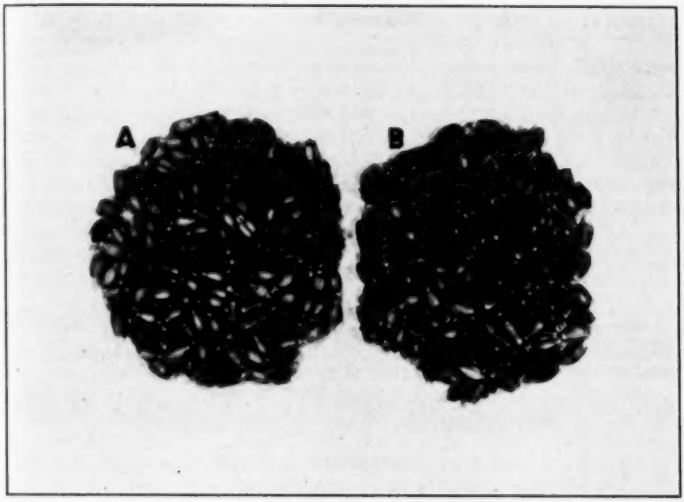


FIGURE 2.—*A*, Wheat from plots of rusted Fulhard plants. High percentage of yellow-berry kernels, a condition here caused directly by leaf rust. *B*, Wheat from control (sulphur-dusted) plots grown under approximately identical conditions except for the absence of leaf rust.

distinctly similar protein and starch contents of the grain from the dusted and nondusted plants.

TABLE 4.—*Effect of leaf rust on relative percentages by weight of vitreous, piebald, and starchy kernels in hard (Fulhard) and semihard (Kawvale) winter-wheat varieties* [a]

| Variety | C.I. no. | Kind of plot | Maximum leaf-rust severity | Kernel type | | |
|---|---|---|---|---|---|---|
| | | | | Vitreous | Piebald | Starchy |
| | | | *Percent* | *Percent* | *Percent* | *Percent* |
| Fulhard | 8259 | Rusted | 100 | 20.4 | 79.6 | |
| | | Control | 10 | 73.9 | 26.1 | |
| Kawvale | 8180 | Rusted | 45 | 10.6 | 60.3 | 29.1 |
| | | Control | 5 | 20.7 | 75.4 | 3.9 |

[a] Determined on 20-gram samples of grain.

Heavy leaf-rust infection had only a small, although consistent, effect on the test weight per bushel of the grain of the susceptible varieties. Gladden and Michigan Amber were most affected, both being reduced in test weight by 1.5 pounds per bushel. In test weight, as in yield, Fulhard was unaffected by the heavy rust in spite of the fact that the rust caused the production of a high proportion of yellow-berry kernels. The test weight of Fultz selection was slightly higher for the rusted plot.

TABLE 5.—*Effect of leaf rust on chemical composition of mature grain of winter-wheat varieties of differing degrees of susceptibility to leaf rust* [a]

| Variety | C.I. no. | Kind of plot | Maximum leaf-rust severity | Dry weight of grain per head [b] | Protein (N×5.7) | Sucrose | Starch | Phosphorus ($P_2O_5$) | Total ash |
|---|---|---|---|---|---|---|---|---|---|
| | | | *Percent* | *Grams* | *Percent* | *Pct.* | *Pct.* | *Percent* | *Pct.* |
| Shepherd | 6163 | Rusted | 100 | 0.868 | 10.25±0.08 | 1.52 | 71.7 | 1.18 | 2.09 |
| | | Control | 13 | 1.070 | 11.90± .10 | 1.73 | 70.0 | 1.21 | 2.17 |
| Michigan Amber | | Rusted | 100 | .685 | 10.20 | 1.12 | 71.4 | 1.14 | 2.07 |
| | | Control | 15 | .886 | 11.53 | 1.49 | 70.5 | 1.13 | 2.00 |
| Gladden | | Rusted | 100 | ..... | 9.45 | 1.39 | 72.7 | 1.12 | 1.96 |
| | | Control | 15 | ..... | 10.81 | 1.53 | 69.6 | 1.13 | 1.95 |
| Fulhard | 8259 | Rusted | 100 | ..... | 10.15 | 1.37 | 71.7 | 1.13 | 1.95 |
| | | Control | 10 | ..... | 12.00 | 1.52 | 70.5 | 1.17 | 2.10 |
| Nittany | | Rusted | 75 | 1.068 | 10.70± .09 | 1.55 | 69.7 | 1.24 | 2.13 |
| | | Control | 10 | 1.128 | 11.62± .10 | 1.65 | 67.9 | 1.23 | 2.13 |
| Purkoff | | Rusted | 65 | ..... | 10.27 | 1.32 | 71.3 | 1.09 | 1.95 |
| | | Control | 8 | ..... | 11.38 | 1.55 | 70.8 | 1.11 | 1.98 |
| Kawvale | 8180 | Rusted | 45 | ..... | 10.19 | 1.29 | 72.2 | 1.12 | 1.87 |
| | | Control | 5 | ..... | 10.58 | 1.43 | 70.6 | 1.09 | 1.88 |
| Fultz selection | 11512 | Rusted | 7 | .913 | 10.90± .09 | 1.21 | 68.7 | 1.14 | 1.94 |
| | | Control | 1 | .876 | 11.06± .09 | 1.25 | 69.0 | 1.16 | 2.01 |

[a] Determined on the threshed grain from each of 4 replicated plots of Shepherd, Nittany, and Fultz selection, on a composite sample of threshed grain from 4 replicated plots of Gladden, Fulhard, Purkoff, and Kawvale and on threshed grain from duplicate plots of Michigan Amber.

[b] Determined on the oven-dry basis on a composite sample of 100 ripe heads from 4 replicated plots of Shepherd, Nittany, and Fultz selection and from duplicate plots of Michigan Amber.

## EFFECT OF LEAF RUST ON CHEMICAL COMPOSITION OF GRAIN AND COMBINED CULMS AND LEAVES

### METHODS

A chemical study was made on the kernels and combined culms and leaves of three varieties, including the susceptible Shepherd, the moderately susceptible Nittany, and the highly resistant Fultz selection, under conditions of severe and light leaf-rust infection. A similar study of the effects of different degrees of leaf-rust severity was made on the susceptible variety, Michigan Amber. In these studies the plants were sampled for analyses when in the early milk, late milk, and dough stages. In addition, analyses were made of the threshed grain of the above-mentioned varieties together with that of Gladden, Fulhard, Purkoff, and Kawvale, also grown under conditions of severe and light leaf-rust infection. The seeding methods and the rust-control methods are described above.

Samples for analysis consisted of 100 tillers selected from the replicated plots, except on June 16, when they consisted of culms and leaves of only 50 tillers and heads of 100 tillers. Through error only 75 heads of Nittany were sampled on that date. Only the larger tillers that appeared to be in a uniform stage of development were selected. The culms were clipped as close to the ground as possible. A proportionate part of the 100 tillers of each sample was taken throughout the central 16 feet of one row in each of the replicated plots. In order to avoid the exceptionally rank plants at the row ends, no tillers were taken from either end of the rows for a distance of 1 foot. Outside rows only, except for Michigan Amber, were sampled, the inner two rows being reserved for determination of final yield and composition of the mature grain. Thus, each of the rows sampled in the early milk, late milk, and dough stages was bordered on one side by a row of the same variety and on the opposite side by that of another. This arrangement was similar in both the control and rusted plots. In the Michigan Amber series inner rows of the plots were available for taking the samples for chemical analyses.

The head samples clipped from the culms in the laboratory immediately after collections were made were dried in a vacuum oven at 70° C. When dry, the kernels were separated from the chaff by hand. The culms and leaves were weighed and finely chopped immediately after gathering. Representative samples were then taken for analysis and dropped into boiling alcohol containing calcium carbonate. At the fully ripe stage only the threshed grain was analyzed.

The samples of combined culms and leaves were analyzed for content of nitrogen, reducing sugars, sucrose, and starch. The same determinations were made on the kernels with the addition of determinations of phosphorus and total ash. Nitrogen was determined by the Kjeldahl method, phosphorus by a modification of the Neumann-Pemberton volumetric method, reducing sugars by a combination of the methods of Quisumbing and Thomas and of Bertrand, sucrose by the invertase method, and starch by the takadiastase method. The total nitrogen of the grain has been reported as protein ($N \times 5.7$). Qualitative tests indicated the absence of nitrate nitrogen in all the materials analyzed. It is realized that the small percentages of material of the culms and leaves, determined as starch by the takadiastase method, may represent levulosans rather than true starch. Since the results of sugar and starch determinations on the immature kernels were very irregular and unsatisfactory, probably owing to enzymatic action occurring during the drying process of the heads, they are not reported here. All analytical data reported in this paper have been calculated on the dry-weight basis.

The probable errors for protein content were determined by the deviation-of-the-mean method (*5*) on the four replicated plots of Shepherd, Nittany, and Fultz selection in both the control and rusted series.

## GRAIN

### Protein

The outstanding finding of the chemical study is that leaf rust, on susceptible or partly susceptible wheat varieties, markedly reduced the protein content of the grain. This held equally in the soft red winter wheats, as represented by the varieties Shepherd, Michigan Amber, and Gladden, in the hard red winter variety, Fulhard, and in the semihard varieties, Purkoff and Kawvale, as shown in tables 5, 6, and 7, and figures 1, 3, and 4.

This reduction in protein appeared to be in approximate proportion to the severity of the rust on the plants. It was demonstrated with Michigan Amber, in which the different degrees of severity of rust at maturity were produced by beginning the dusting treatment of different plots at successively later dates, i.e., at the jointing, booting, and flowering stages, while others were not dusted. The plots that became infected with leaf rust to the extent of 15, 52, and 100 percent of relatively late development and 100 percent of earlier development produced grain containing, respectively, 11.53, 11.00, 10.53, and 10.20 percent of protein (table 7 and fig. 1). That the percentage of protein is affected in approximate proportion to the rust severity also is shown by the other varieties, the protein content of the more susceptible varieties being very distinctly reduced, while that of the resistant varieties was not appreciably affected. The effect on the moderately susceptible and moderately resistant varieties was intermediate between these two extremes. Thus, the protein content of

Shepherd (rusted 100 percent), Nittany (rusted 75 percent), and Fultz selection (rusted only 7 percent) was 1.65, 0.92, and 0.16 percent less, respectively, than that of the controls (table 5).

The differences in protein content of the grain from the rusted and control plants became pronounced sometime between the late milk and dough stages (tables 6 and 7 and fig. 3). It was immediately before this period that the rust infection reached its maximum on most of the varieties. Small differences in percentages of protein due to leaf rust were consistently found in the grain of susceptible varieties in the late milk stage and became progressively greater as

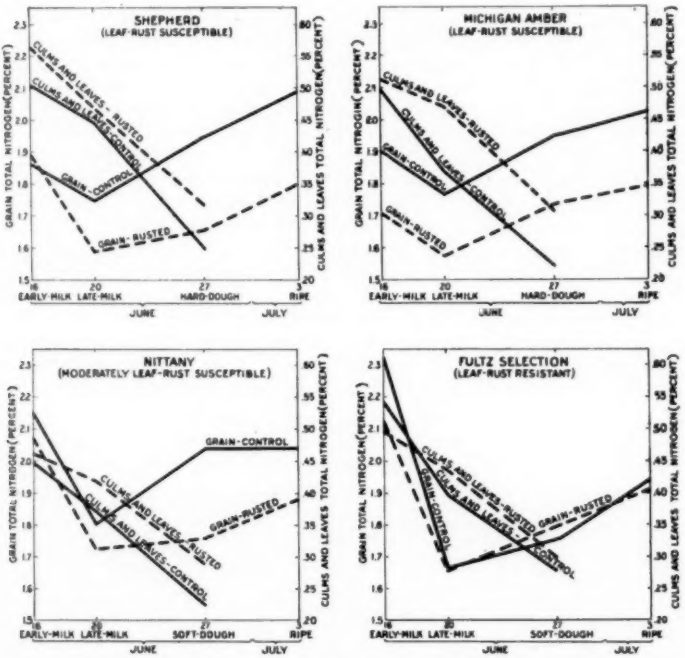


FIGURE 3.—Percentage of total nitrogen present in grain and vegetative portions of rusted and control wheat plants from the early milk stage to maturity.

the grain approached maturity. As shown in figure 3, the protein percentage of the grain of all varieties sampled in the earlier stages fell greatly between the early milk and late milk stages for both the rusted and control plants. Thereafter the proportion as well as the total quantity (fig. 4) of protein per head rose in both the rusted and control plants. This is similar to the findings of Brenchley and Hall (*2*), Thatcher (*21*), Woodman and Engledow (*22*), Kiesselbach (*12*), and Saunders (*18*), who studied chemically the development of the normal kernel. It is to be noted, however, that the drop in the percentage of protein is not so great in grain from control plants as in grain from rusted plants, and that the subsequent rise is greater in the former.

This reduction in the proportion of protein was associated with a similar reduction in the total dry weight of the grain per head and in

acre yield for all susceptible varieties except Fulhard, where no significant change in yield was induced by the rust (fig. 2). The accumulation of nitrogenous materials in the kernels was continuous from the early milk stage until maturity $\phi$ in both the rusted and control plants, but it was more rapid and greater in the controls (fig. 4).

The total acre production of protein is of interest, especially in the hard wheats, in which the relative value of the crop is in a large degree

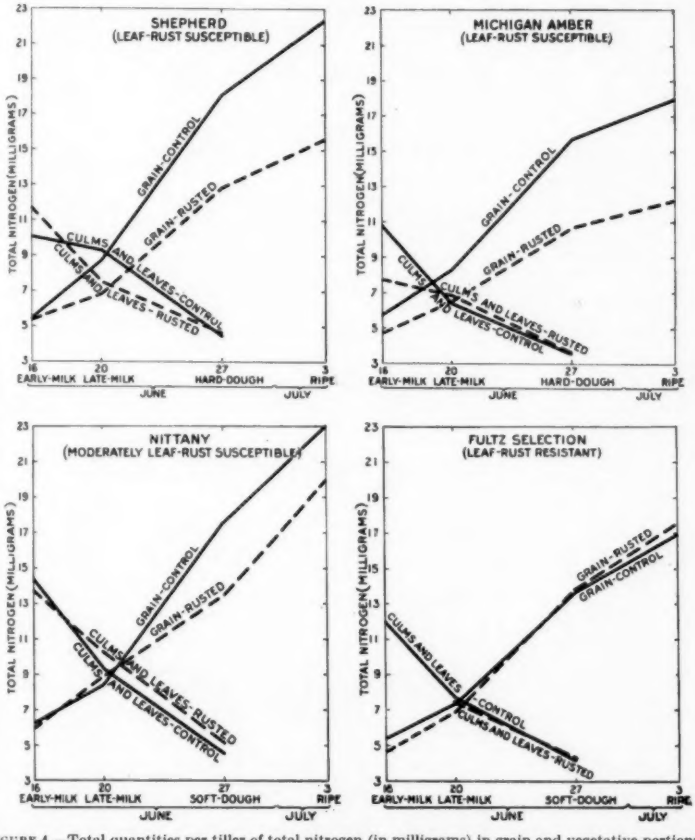


FIGURE 4.—Total quantities per tiller of total nitrogen (in milligrams) in grain and vegetative portions of rusted and control wheat plants, from early milk stage to maturity. (Since the dry weight of the sample from the rusted plot of Michigan Amber was accidentally not determined on June 1, the value given on that date is for the plots first dusted in the flowering stage, which were also severely rusted.)

dependent on the protein content. Since both the quantity of grain and the proportion of protein were reduced by leaf rust in most of the susceptible varieties, the acre yield of protein, which is a function of the two, was greatly affected. In Shepherd and Michigan Amber the yields of protein were reduced 38.3 and 30.2 percent, respectively (tables 1 and 2). In Fulhard, in which yield of grain was not reduced by rust, the protein yield was reduced 14.6 percent.

TABLE 6.—*Effect of leaf rust on chemical composition at different stages of maturity of the kernels and vegetative parts of winter-wheat varieties of differing degrees of susceptibility to leaf rust*

| Variety and C. I. no. | Date of sampling | Stage of maturity of kernels | Kind of plot | Leaf-rust severity | Living leaves per tiller | Grain [a] | | | | Culms and leaves [b] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Dry weight per head | Protein (N×5.7) | Phosphorus $P_2O_5$ | Total ash | Average dry weight per tiller | Total nitrogen | Reducing sugars | Sucrose | Total sugars | Starch |
| | | | | *Percent* | *Number* | *Grams* | *Percent* | *Percent* | *Percent* | *Grams* | *Percent* | *Percent* | *Percent* | *Percent* | *Percent* |
| Shepherd, 6163 | June 16 | Early milk | Rusted | 95 | 2 | 0.287 | 10.77 | 1.09 | 2.41 | 2.078 | 0.563 | 3.09 | 13.39 | 16.48 | 1.38 |
| | | | Control | 10 | 2½ | .292 | 10.61 | 1.02 | 2.34 | 1.993 | .504 | 2.84 | 16.86 | 19.70 | 1.44 |
| | June 20 | Late milk | Rusted | 100 | 1½ | .431 | 9.06 | 1.01 | 2.18 | 1.599 | .470 | 4.09 | 9.52 | 13.60 | 1.41 |
| | | | Control | 13 | 2½ | .513 | 9.96 | 1.05 | 2.20 | 2.081 | .446 | 3.53 | 13.58 | 17.11 | 1.46 |
| | June 27 | Hard dough | Rusted | 100 | 0 | .777 | 9.44 | 1.05 | 1.99 | 1.468 | .316 | 3.41 | 1.54 | 4.95 | 1.13 |
| | | | Control | 13 | ½ | .930 | 11.09 | .99 | 1.98 | 1.786 | .248 | 6.04 | 4.63 | 10.67 | 1.23 |
| | July 3 | Mature | Rusted | 100 | 0 | .868 | 10.25±0.08 | 1.18 | 2.09 | | | | | | |
| | | | Control | 13 | 0 | 1.070 | 11.90±0.10 | 1.21 | 2.17 | | | | | | |
| Nittany | June 16 | Early milk | Rusted | 50 | 2 | .288 | 11.79 | 1.15 | 2.66 | 2.972 | .462 | 3.56 | 17.68 | 21.25 | 1.32 |
| | | | Control | 3 | 2½ | .289 | 12.25 | 1.13 | 2.67 | 3.205 | .447 | 3.49 | 19.49 | 22.98 | 1.35 |
| | June 20 | Late milk | Rusted | 75 | 1 | .513 | 9.86 | 1.11 | 2.32 | 2.458 | .419 | 3.97 | 13.98 | 17.95 | 1.40 |
| | | | Control | 10 | 2 | .465 | 10.29 | 1.11 | 2.43 | 2.494 | .373 | 4.46 | 17.84 | 22.29 | 1.65 |
| | June 27 | Soft dough | Rusted | 75 | 0 | .765 | 10.05 | 1.12 | 2.13 | 1.778 | .290 | 5.92 | 3.76 | 9.68 | .94 |
| | | | Control | 10 | ½ | .861 | 11.62 | 1.10 | 2.10 | 2.072 | .223 | 7.45 | 8.49 | 15.94 | 1.09 |
| | July 3 | Mature | Rusted | 75 | 0 | 1.098 | 10.70±0.09 | 1.24 | 2.13 | | | | | | |
| | | | Control | 10 | 0 | 1.128 | 11.62±0.10 | 1.23 | 2.13 | | | | | | |
| Fultz selection, 11512 | June 16 | Early milk | Rusted | 4 | 2½ | .220 | 12.09 | 1.18 | 2.31 | | .495 | 2.44 | 14.54 | 16.98 | 1.43 |
| | | | Control | 1 | 2½ | .232 | 13.25 | 1.06 | 2.33 | 2.221 | .540 | 2.34 | 14.79 | 17.12 | 1.38 |
| | June 20 | Late milk | Rusted | 7 | 1½ | .414 | 9.46 | 1.06 | 2.24 | 1.690 | .437 | 3.37 | 11.52 | 14.89 | 1.31 |
| | | | Control | 1 | 2½ | .440 | 9.48 | 1.02 | 2.17 | 1.919 | .398 | 3.31 | 13.40 | 16.71 | 1.54 |
| | June 27 | Soft dough | Rusted | 7 | 0 | .768 | 10.23 | 1.01 | 1.93 | 1.424 | .300 | 3.58 | 3.26 | 6.84 | 1.21 |
| | | | Control | 1 | 1 | .778 | 9.98 | 1.02 | 1.98 | 1.493 | .277 | 4.35 | 4.73 | 9.08 | 1.20 |
| | July 3 | Mature | Rusted | 7 | 0 | .913 | 10.90±0.09 | 1.14 | 1.94 | | | | | | |
| | | | Control | 1 | 0 | .876 | 11.06±0.09 | 1.16 | 2.01 | | | | | | |

[a] Determined in the premature stages on a composite sample of kernels of 100 heads collected partly from each of 4 replicated plots of each variety, except for Nittany on June 16, when 75 heads were analyzed, and in the mature stage on separate samples of the threshed grain from each of 4 replicated plots of each variety.

[b] Determined on a composite sample of 100 tillers (culms and leaves combined) collected partly from each of 4 replicated plots of each variety, except for June 16, when 50 tiller samples were used.

TABLE 7.—*Effect of leaf rust in different degrees of severity on chemical composition of kernels and vegetative parts of Michigan Amber wheat at different stages of maturity*

| Stage of plant maturity at beginning of dusting | Stage of maturity of kernels | Date of sampling | Leaf-rust severity [a] | Estimated average living leaves per tiller | Grain [b] | | | | Culms and leaves [c] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Dry weight per head | Protein (N×5.7) | Phosphorus ($P_2O_5$) | Total ash | Dry weight per tiller | Total nitrogen | Reducing sugars | Sucrose | Total sugars | Starch |
| | | | *Percent* | *Number* | *Grams* | *Percent* | *Percent* | *Percent* | *Grams* | *Percent* | *Percent* | *Percent* | *Percent* | *Percent* |
| Nondusted | Early milk | June 16 | 100 | 1 | 0.279 | 9.70 | 1.06 | 2.25 | 0.511 | 2.55 | 13.04 | 15.59 | 1.25 | |
| Flowering | do | do | 100 | 1 | .255 | 9.58 | 1.08 | 2.33 | 1.559 | .497 | 2.60 | 12.69 | 15.29 | 1.49 |
| Booting | do | do | 52 | 1½ | .309 | 9.86 | 1.01 | 2.22 | 2.052 | .484 | 2.56 | 15.81 | 18.37 | 1.51 |
| Control (jointing) | do | do | 13 | 2½ | .303 | 10.81 | 1.02 | 2.36 | 2.164 | .495 | 2.58 | 17.54 | 20.13 | 1.38 |
| Nondusted | Late milk | June 20 | 100 | 0 | .413 | 8.96 | 1.02 | 2.17 | 1.460 | .469 | 3.88 | 8.04 | 11.92 | 1.23 |
| Flowering | do | do | 100 | 1 | .420 | 9.00 | 1.02 | 2.22 | 1.451 | .438 | 3.79 | 9.41 | 13.20 | 1.40 |
| Booting | do | do | 52 | 1 | .446 | 9.50 | 1.00 | 2.10 | 1.607 | .349 | 3.63 | 12.35 | 15.99 | 1.54 |
| Control (jointing) | do | do | 15 | 1½ | .459 | 10.05 | 1.00 | 2.12 | 1.733 | .368 | 3.41 | 14.89 | 18.29 | 1.38 |
| Nondusted | Hard dough | June 27 | 100 | 0 | .614 | 9.88 | 1.10 | 2.07 | 1.195 | .305 | 2.55 | .84 | 3.39 | 1.19 |
| Flowering | do | do | 100 | 0 | .631 | 10.01 | 1.06 | 1.99 | 1.129 | .287 | 2.94 | 1.21 | 4.15 | 1.17 |
| Booting | do | do | 52 | 0 | .703 | 10.55 | 1.04 | 1.99 | 1.167 | .269 | 4.28 | 3.10 | 7.38 | .96 |
| Control (jointing) | do | do | 15 | 1 | .808 | 11.09 | 1.01 | 1.99 | 1.616 | .220 | 6.17 | 4.50 | 10.68 | 1.10 |
| Nondusted | Mature | July 3 | 100 | 0 | .685 | 10.20 | 1.14 | 2.07 | | | | | | |
| Flowering | do | do | 100 | 0 | .725 | 10.53 | 1.15 | 2.11 | | | | | | |
| Booting | do | do | 52 | 0 | .788 | 11.00 | 1.15 | 2.08 | | | | | | |
| Control (jointing) | do | do | 15 | 0 | .886 | 11.53 | 1.13 | 2.00 | | | | | | |

[a] On June 16, 100 percent of leaf rust was present on both the nondusted plots and those dusted beginning at the flowering stage; the epiphytotic developed earlier and therefore more severely on the nondusted plots.

[b] Determined in the premature stages on a composite sample of kernels of 100 heads collected partly from each of duplicate plots and in the mature stage on separate samples of the threshed grain from duplicate plots.

[c] Determined on composite samples of 100 tillers (leaves and culms combined) collected partly from each of duplicate plots except for June 15, when 50 tiller samples were used.

### Starch and Sucrose

The greater portion of the dry weight of the mature grain consisted of starch, which varied from approximately 69 to 73 percent in the various samples analyzed. Since protein and starch are two of the major constituents of the grain, one might expect to find, as was found, that a certain fluctuation in one would be reflected in a reverse fluctuation in the other. In the susceptible and moderately susceptible varieties, from approximately 1 to 3 percent more starch was consistently found in the less proteinaceous grain of the rusted plants than in that from the control plants (table 5). On the other hand, very little difference was found between the starch content of the grain from rusted and control plants of the resistant Fultz selection. Here again there is evidence that the sulphur dusting did not affect the composition when leaf rust was not a factor.

The percentage of sucrose in the grain, in contrast to that of the starch, was consistently lower for the rusted than for the control plants, although but small percentages were found in either case. In general, the most susceptible varieties showed the greatest differences. In Michigan Amber the sucrose content of the mature grain was 1.49, 1.45, 1.33, and 1.12 percent for plants ranging from light to very severe in leaf-rust infection.

### Phosphorus and Total Ash

Leaf rust produced no significant changes in the phosphorus content of any variety in either the immature or mature grain. The same can be said of the total ash content, namely, that, although the varieties differed, the grain from the rusted plants of a given variety was very similar to that of the control plants of that variety. Since the head and acre yields of the heavily rusted plants were considerably reduced, the total quantities of ash and phosphorus per head were, of course, greater in the control plants.

## COMBINED CULMS AND LEAVES

### Total Nitrogen

In contrast to the grain, the vegetative portions, i.e., the culms and leaves, of the rusted plants contained a distinctly higher proportion of nitrogenous components than did corresponding parts of the control plants. Thus, the rusted plants, although higher than the control plants in respect to the proportion, and usually the total quantity, of nitrogenous materials, produced a distinctly less nitrogenous grain than did the control plants (tables 5, 6, and 7 and figs. 3 and 4).

This difference in nitrogen content of the culms and leaves, caused by leaf rust, was evident in the susceptible varieties in the early milk stage and had become more pronounced at the latest period of sampling, when the kernels were in the dough stage. The greatest difference at the dough stage was observed in Michigan Amber, in which the most heavily rusted plants contained 0.305 percent of total nitrogen in the culms and leaves, as contrasted with 0.22 percent in the control plants, an actual retention of a 38.6 percent greater concentration of nitrogen in the rusted than in the control plants. In Fultz selection, the most resistant variety, the concentration of

nitrogen in the rusted plants exceeded that of the control plants by only 8.3 percent. This indicates that the rust was primarily responsible for the much greater differences between the rusted and control plants of the susceptible varieties.

At the hard-dough stage the plants of Michigan Amber bearing different severities of leaf-rust infection presented a graded series as regards percentage of total nitrogen content of the culms and leaves (table 7). With slight deviations this also held in the earlier stages.

The total quantity of nitrogen retained in the culms and leaves of the rusted tillers was usually greater than that in the control tillers, even though these parts of the rusted tillers actually contained less dry matter (fig. 4 and tables 6 and 7). In contrast, the grain from these rusted tillers contained much lower quantities of total nitrogen than did that from control tillers.

In the analysis of the culms and leaves the rust mycelium and spores that still adhered to the plants were necessarily analyzed with the host-plant tissues. Since this nitrogenous material was taken from the host plant by the fungus, it seems reasonable to consider it with that of the rusted host-plant tissues. From these studies there is no means of knowing whether the excess of nitrogen in the vegetative parts of the rusted plant as compared with the control plants was largely incorporated in the fungus or held in the host-plant tissues through a possible interference with translocation or metabolism.

### Sucrose

As judged from the nearly rust-free control plants and the resistant varieties (tables 6 and 7) the sucrose content of the culms and leaves normally dropped from a range of about 15 to 20 percent at the early milk stage to from one-fourth to one-half of this content at the dough stage. The rusted plants of the several susceptible varieties showed a much more decided drop in this constituent (tables 6 and 7). For example, the sucrose content of leaves and culms of the Michigan Amber control plants fell from 17.54 percent in the early milk stage on June 16 to 4.50 percent in the hard-dough stage on June 27. During the same period the rusted plants showed a drop from 13.04 to 0.84 percent in sucrose. This reduction of sucrose in Michigan Amber varied directly with the abundance of rust, as shown in table 7, which also shows that plants ranging in rust infection from light to heavy had a sucrose content of 4.50, 3.10, 1.21, and 0.84 percent at the hard-dough stage. The differences between the sucrose content of lightly and heavily rusted plants had already become evident at the early milk stage on June 16. As a check on the sulphur-dusting method of studying the effect of leaf rust on the host, the extremely resistant Fultz selection shows only a slight effect of the rust, or possibly that of the sulphur, on the sucrose content of the vegetative parts. Since the nondusted (rusted) plot of this variety had 7 percent of rust, the small difference of 1.47 percent between the rusted and control plants may be largely accounted for as an effect of the rust attack rather than as a direct effect of the sulphur on the plant metabolism.

### Reducing Sugars

In contrast to the sucrose and as judged by the control plants and the resistant Fultz selection, reducing sugars in the culms and leaves normally tended to accumulate as the plants matured. This increase

was approximately 100 percent from the early milk to the dough stages (tables 6 and 7). Unfortunately, no analyses were made of the vegetative parts when fully ripe. Heavily rusted plants failed to show this accumulation of reducing sugars, the percentage content remaining about the same from the early milk to the dough stages. The graded series of rust severity on Michigan Amber (table 7) produced a distinct gradient in the reducing-sugar content of the culms and leaves, with percentages of 6.17, 4.28, 2.94, and 2.55 resulting on June 27 from leaf-rust infections, ranging from light to very heavy.

### Starch

Starch, or the materials determined as starch by the described method of determination, is a relatively unimportant constituent of the culms and leaves of the wheat varieties studied, composing only about 1 to 1.5 percent of the total dry weight. Leaves and culms of the control plants, as they matured, showed no definite changes in this constituent. Leaf rust apparently has little or no important effect on these materials. It is recognized that the materials here described probably represent levulosans instead of starch.

## DISCUSSION

The findings reported in this paper add further evidence to those supplied by Kightlinger and Whetzel (*13*), Mains (*14*), and Johnston (*10*) that leaf rust is a very important factor affecting yield of winter wheats in the United States. The losses of more than 28 percent in the yield of Shepherd and 20 percent in that of Michigan Amber are probably fairly representative of the destructiveness of a severe leaf-rust epiphytotic on susceptible winter wheats in Indiana. Such epiphytotics are not infrequent there, and most of the commercial varieties are extremely susceptible to this disease.

Fulhard probably was the most severely rusted variety in the test, yet its yield was not reduced. This is the more puzzling because the physical characters and chemical composition of the grain of Fulhard were more greatly affected by the rust infection than those of any other variety in the trial. Salmon and Laude (*17*) reported a similar result with Fulhard in 1929. They found a distinct relationship between leaf-rust resistance and yielding ability in 24 varieties tested for yield. Although Fulhard was one of the varieties most severely attacked by leaf rust, it produced the highest yield of the entire group.

No satisfactory explanation can be given to account for the apparent depression of yield in the sulphur-dusted plots of the Fultz selection, the highly leaf-rust-resistant variety. All of the replicated plots of this variety that were not dusted were located beside a 2-foot nursery path but protected from border effect by one guard row of the same variety. It is possible that border effect extended into the harvested rows of this variety, thus increasing the yield. However, a similar but less pronounced depression of yield in the sulphur-dusted plots occurred also in the semiresistant variety, Kawvale, in which there was no possible border effect. It is also possible that, since leaf rust was not a factor in the yield of the rusted plots, a possible injurious effect of the sulphur may have become apparent in the yield of the control plots.

Very little study has been made of the effect of cereal rusts on the composition of the infected host plants and the grain produced by them. The opinion is commonly held that both stem rust and leaf rust tend to increase the protein percentage in the wheat grain. But few experimental data have been found in the literature to support this view, and these cannot be accepted without question. In 1889 Harper (*5*), in an analysis of shrunken, "rusted or frosted", wheat from Minnesota, found that it contained more than 2 percent more protein than did the well-filled grain. Shutt (*19*), in Manitoba, and Snyder (*20*), in Minnesota, analyzed both the straw and grain of severely stem-rusted and stem-rust-free wheat plants. Both found that the percentage of crude protein was much higher in the straw and grain of rusted plants than in the straw and grain of nonrusted plants. Shutt reported that the grain from rusted wheat contained 13.69 percent of protein, as compared to only 10.50 percent in the rust-free wheat. Snyder found that the grain from heavily rusted plants contained 16.37 percent of protein as compared to 13.34 percent in the rust-free plants. Shutt found three times as much crude protein in rusted straw as in rust-free straw. Snyder's findings relative to the protein content of the straw accorded with those of Shutt. Ince (*9*), in North Dakota, found rusted straw much higher in protein than was nonrusted straw.

If stem rust tends to increase the protein content of the grain, as these data seem to show, the effect is directly contrary to that of leaf rust, which undoubtedly decreases the protein content. Neither Shutt nor Snyder states just how rusted and nonrusted plants were obtained from the one field sampled in each case. It would seem likely that under conditions of a severe stem-rust epiphytotic it would be necessary to take rusted and nonrusted samples from relatively low and high elevations, respectively, of the single field, which, if done, might alone account for the variations in protein content of the grain, as shown by Newton and Malloch (*16*).

Contrary to the findings of both Shutt and Snyder, Headden (*8*), working in Colorado, concluded that a heavy stem-rust epiphytotic in 1915 was responsible for the low protein content of certain wheat varieties grown that year as compared with the protein content of the same varieties grown in 1913, when rust was not an important factor. His conclusions were based on the fact that the two crops were grown on the same piece of land and in both years had received identical irrigation and fertilization. He found that a greater percentage of the total nitrogen of the plant remained in the straw in the rust year of 1915 than was the case in 1913. This was in general accord with the results of Shutt and Snyder. Headden attributes this condition to the interference of the rust in the translocation of nitrogen into the head. Thus, his interpretation of the effect of stem rust on the movement of nitrogenous compounds in the plant is similar to the writers' findings for leaf rust. However, it is possible that factors other than stem rust, not under control in Headden's experiments, may have been responsible for the low-protein grain of 1915. Mangels and Sanderson (*15*) concluded, on an apparently more secure basis, that stem rust tends to reduce the protein content of the grain. In 1922, when stem rust was severe at Fargo, N.Dak., they found that the rust-susceptible varieties produced grain "quite low" in protein content and that the grain of the most susceptible

varieties was lowest in protein. Since the varieties studied had not shown these differences in protein percentages in years when stem rust was not a factor, they believed that stem rust was responsible for them.

Leaf rust is mentioned neither by Headden nor by Mangels and Sanderson. If present it might have been largely responsible for the low protein content attributed to stem rust. The literature reviewed on the subject apparently affords no sound basis for an opinion regarding the true effect of stem rust on the protein content of the wheat kernel.

In 1927, Broadfoot (*3*), working in Minnesota on spring wheat grown in leaf-rusted plots and plots in which the rust was partly controlled by sulphur dusting, concluded that leaf rust was responsible for a slight increase in protein content and a slight shriveling of the grain. This is contrary to the conclusion of the writers. Because of the irregularity in the protein content of the grain in the different plots of each of Broadfoot's series of dusted and nondusted plots, the small average difference (0.36 percent) in protein, which was found between the two series, appears insignificant. The shriveling of the kernels that he reports is suggestive of injury by heat and drought or by stem rust rather than by leaf rust, as shown by Mains (*14*) and Johnston (*10*) and by the writers in the present paper. In the writers' investigations at La Fayette, Ind., they found that yield losses of more than 25 percent caused by leaf rust have not been attended by any evident shriveling of the kernels.

The ultimate result of severe leaf-rust infection was the retention of a greater proportion, as well as a greater quantity, of the total nitrogenous materials of the plant within the leaves and stems of the rusted plants than within those of the control plants. The writers were unable to determine whether the retention of these materials in the vegetative parts is to be accounted for primarily as a direct result of their utilization by the fungus, as a result of direct interference of the fungus in metabolism or translocation of the nitrogenous materials, or as a combined result of these factors, because the nitrogen in the rust spores and in the mycelium cannot be determined separately from that within the host tissues. The excess quantity of nitrogen per tiller present at any one time in the rusted culms and leaves, as compared with that in the control plants, does not equal the deficiency of total nitrogen per head in the mature grain from rusted plants. This fact may be partly accounted for by the continuous loss of nitrogenous material from the rusted plants in the form of urediospores that are scattered by the wind or that often fall to the ground in quantities sufficient to give the soil a distinctly brick-red cast. It is also possible that nitrogenous materials may be more readily leached from the early-killed leaves of rusted plants than from the longer-lived foliage of control plants.

The reduced nitrogen percentage in the kernels of the rusted plants was invariably associated with an increased percentage of starch. However, the fungus reduces the total quantity of nonnitrogenous material available for storage as starch in the kernels, as is definitely shown in the total quantity of starch per head laid down in the rusted and nonrusted plants. The increased percentage of starch in the kernels of the rusted plants must be due to a differential effect of the fungus on the supply of these two classes of elaborated plant food.

Evidently, the impaired photosynthetic activity of the rusted plant, due to early death of the leaves, is not proportionately so great as is the effect of the fungus in limiting the nitrogenous materials available for storage as protein in the grain. The production of increased numbers of yellow-berry and starchy kernels by rusted plants as compared with control plants is definitely correlated with a decreased proportion of protein to starch within the kernel. The limitation of nitrogen by the rust seems to result directly in the production of a softer grain of lighter color. In the hard wheats the proportion of yellow-berry kernels is increased by the leaf-rust infection, whereas in a soft wheat a more uniformly starchy kernel results.

The quality of soft wheats may not be seriously impaired by these effects of leaf rust, since low-strength flours of at least moderately low protein content are desirable in the production of crackers, pastry, and biscuits, for which the soft-wheat flours are primarily used.[5] The effect of a reduced protein-percentage content and an increased proportion of yellow-berry kernels, however, would constitute a serious impairment in the quality of hard red winter wheat, where high strength of flour and high protein content are desirable. At La Fayette, Ind., the marked effect of leaf rust on the quality of Fulhard wheat, a hard winter type, suggests that a similar effect may occur in the hard-winter-wheat region. This may occur especially in years when nitrogen is a limiting factor.

The extreme variation in the severity of leaf rust in different years is apparently an important contributing factor in the variability in the quality of the winter wheats from year to year. The development and use of winter-wheat varieties resistant to leaf rust would eliminate this factor as a cause of variability.

So far as known, neither agronomists nor physiologists have considered leaf rust a factor in the yellow-berry problem, although there has been marked diversity of opinion regarding the relative importance of the influences of heredity and of climatic and fertility conditions on the expression of yellow berry. Bolley (*1*) has ascribed the cause of yellow berry, as well as black point and other kernel diseases, to direct infection with fungi. Later workers, Headden (*7*) and Jones and Mitchell (*11*), have shown that yellow berry is clearly distinguishable from other kernel blemishes caused by fungous infection and that it may be controlled by cultural practice. It should be understood that leaf rust also may produce the yellow-berry condition but does so through its effect on the whole plant and not by direct infection of the grain.

The significance of leaf-rust infection of experimental plots has received little recognition in studies of the normal development of the wheat kernel. Most workers have failed to mention its presence or absence. Saunders (*18*) has described what apparently was a moderately severe leaf-rust epiphytotic, which was "rather bad" in his experimental plots 11 days before the grain ripened. However, he dismissed leaf rust as a significant factor in his chemical studies of the development of the kernels. This omission by investigators of the chemistry of kernel development undoubtedly is due to the very slight effect of leaf rust on the size and shape of the grain in contrast to the drastic effect of stem rust, which they could not disregard. A

[5] Samples of the wheats from the experiments reported in this paper have been experimentally milled, and baking studies on the flours are in progress.

record of the occurrence of leaf rust might have helped to explain in many cases the great diversity of results in studies of normal development of the wheat kernel.

## SUMMARY

A study was made at La Fayette, Ind., in 1931, of the effects of a severe leaf-rust epiphytotic on the yield and physical characters of the grain and on the chemical composition of the grain and plants of (1) seven varieties of winter wheat of differing degrees of leaf-rust resistance, and (2) of one very susceptible variety infected with rust in four different degrees of severity. Very lightly rusted control plants were provided for comparison by frequently dusting the plants with sulphur, while variations in the dusting procedure provided a graded series of severities of rust infection.

In the very susceptible varieties, with one exception, reductions in yield of grain ranging from 14.8 to 28.4 percent were associated with heavy infections of leaf rust. The yield of Fulhard was not reduced, even though this variety was severely rusted. In most varieties the losses were approximately proportional to the severity of the rust. The yields of straw and grain were affected alike.

Approximately three-fourths of the grain losses caused by leaf rust resulted from a reduction in the number of kernels per head, and the remainder from a reduction in weight per kernel.

The most marked effect of leaf rust on the physical characters of the grain was a greatly increased proportion of yellow-berry or piebald kernels in the hard and semihard varieties, and the production of kernels more uniformly soft or starchy in the soft wheats. Even under almost maximum severity of rust at flowering time no shriveling of the kernels occurred. The test weight per bushel was slightly, though consistently, reduced by leaf rust.

The percentage of protein in the grain of susceptible varieties of both hard and soft winter wheats was very significantly reduced by severe leaf-rust infection. The effects were less pronounced in the more resistant varieties. The same was true of a susceptible variety when leaf rust was partly controlled.

In contrast to the grain, the combined culms and leaves of the rusted plants contained higher percentages of total nitrogen, and, in most cases, greater quantities of total nitrogen per tiller than did those of the control plants.

The starch percentage content of the mature grain varied inversely with the protein percentage, the lower-protein grain from rusted plants being higher in starch than was the grain from control plants. However, because of the reduced number and size of kernels, the total quantity of starch laid down per kernel and head was distinctly reduced by leaf rust.

Sucrose of the mature grain, although a minor constituent, was consistently reduced in concentration by leaf rust.

The culms and leaves of rusted plants at the nearly ripe stages contained lower percentages of both sucrose and reducing sugars than did the control plants.

The percentages of phosphorus and total ash of the grain were not appreciably affected by leaf rust.

Fultz selection, a highly resistant wheat, which was practically unaffected by leaf rust, showed no direct effect of sulphur dusting on the visible characters of the grain or on composition.

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# INHERITANCE OF RESISTANCE TO LOOSE SMUT AND COVERED SMUT IN SOME OAT HYBRIDS[1]

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## INTRODUCTION

In recent years several investigators have obtained extensive data on the inheritance of smut resistance in oat (*Avena*) hybrids. Crosses between oat varieties showing various types of resistance to the smuts were made, and the behavior of the hybrids in the $F_2$ and $F_3$, and, occasionally, in later generations was recorded. The published results have been reviewed in recent papers by Coffman et al. (*3*)[2] and Reed (*14*). The present paper deals with six oat crosses involving varieties that show different degrees of resistance to loose smut (*Ustilago avenae* (Pers.) Jens.) and covered smut (*U. levis* (Kell. and Sw.) Magn.). Data on the reaction of $F_2$ and $F_3$ plants are included.

Since recent investigations have demonstrated a high degree of specialization in both species of oat smuts, it is particularly important, in studies on the inheritance of smut resistance, not only to distinguish between the morphological species *Ustilago avenae* and *U. levis* but to recognize the fact that both species contain specialized races or physiologic forms characterized by differences in their capacity for infection. Reed (*7, 9, 11*) differentiated 9 distinct races of loose smut and 5 of covered smut. More recently, Reed and Stanton (*16*) have described a new race of covered smut distinguished by its capacity for infecting strains of the Fulghum oat. Reed (*15*) has also discovered that this race of smut is capable of infecting Black Mesdag, a variety exceptionally resistant to other known races of loose and covered smuts. In the studies herein described 3 distinct specialized smut races were used for inoculation, 2 belonging to *Ustilago avenae* and 1 to *U. levis*. These specialized races are designated as *U. avenae*-Missouri, *U. avenae*-Fulghum, and *U. levis*-Missouri. Their characteristics have been reported in the publications noted above.

## MATERIALS AND METHODS

The six oat crosses studied were Monarch Selection × Black Mesdag, Richland × Fulghum, Richland × Markton, Markton × Iogold, Markton × Black Mesdag, and Cornellian × Markton. There were two hybrids of each varietal combination. The first-named variety was used as the female parent in all the crosses; there were no reciprocals. The crosses were made in the greenhouse

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[2] Reference is made by number (italic) to Literature Cited, p. 1082.

at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D.C., in the spring of 1927. The parents were the strains of these varieties grown by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

Detailed information as to the origin, economic value, and main characteristics of the varieties used as parents may be found in the literature on the registration and classification of oats (*4*, *17*, *19*, *20*, *21*, *22*). Black Mesdag (C.I.[3] no. 1877) and Markton (C.I. no. 2053) are of special interest because of their high degree of resistance to both species of smuts. Markton also has proved to be a valuable commercial variety in Washington, Oregon, Idaho, and western Montana (*22*). Cornellian (C.I. no. 1242) is a comparatively new, gray-kerneled variety, grown extensively in New York (*6*). Fulghum (C.I. no. 708) is widely distributed in the central and southern oat areas, and Iogold (C.I. no. 2329) and Richland (C.I. no. 787) are extensively grown in the Corn Belt States. Black Mesdag and Monarch Selection (of Etheridge) are black-kerneled varieties of little agronomic value.

Seed from the $F_1$ plants and from the parental strains was sent to the Brooklyn Botanic Garden, Brooklyn, N.Y., in the spring of 1928. A detailed study of the inheritance of smut resistance in these hybrids was conducted in 1928, 1929, 1930, and 1931. Three sets of seed were taken from each $F_1$ plant of the different crosses. One set was inoculated with *Ustilago avenae*-Missouri, the second with *U. avenae*-Fulghum, and the third with *U. levis*-Missouri. The three sets of $F_2$ plants were grown in 1928, together with the parental varieties similarly inoculated. In 1929 there were grown additional $F_2$ plants of some of the crosses inoculated with *U. avenae*-Missouri and *U. levis*-Missouri.

The experiments with the $F_3$ generation of the hybrids were conducted under field conditions in 1929, 1930, and 1931. Usually, two sets of seed were taken from each $F_2$ plant, one set being inoculated with *Ustilago avenae*-Missouri and the other with *U. levis*-Missouri. In one of the crosses, however, one set of the $F_3$ progenies was inoculated with *U. avenae*-Fulghum instead of with *U. levis*-Missouri. The $F_3$ progenies were grown together with the parental varieties similarly inoculated.

The methods of inoculating and germinating the seed have been fully described by Reed (*14*). The usual procedure was to remove the hulls, inoculate the seed with dry chlamydospores, and allow them to germinate in sand with a water content of approximately 20 percent at a temperature of about 20° C. When the seedlings were 1 or 2 inches high they were transplanted to the field.

The profound influence of environmental factors on the infection of oats with the smuts is recognized by careful investigators. The low percentages of infection reported by a few workers may be accounted for by neglect to control soil moisture, soil temperature, and other conditions. The presence of hulls, especially in connection with certain external factors, has an important influence on the number of smutted plants. In the present studies the influence of such factors was largely eliminated, and the results obtained must be accounted for on the basis of inherited factors governing resistance.

[3] C.I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

## EXPERIMENTAL DATA

### $F_2$ PLANTS

Table 1 presents the summarized data on the reaction of the inoculated $F_2$ plants of the six hybrids and the data on the inoculated parental varieties grown with the $F_2$ plants and the $F_3$ progenies.

In general, the data in table 1 conform to expectation, but there are some exceptions that should be pointed out. The combination Monarch Selection × Black Mesdag is of interest. Monarch Selection is very susceptible to *Ustilago avenae*-Missouri but highly resistant to *U. levis*-Missouri, whereas Black Mesdag is highly resistant to both smuts. The $F_2$ results from this cross, as shown in table 1, might be interpreted as indicating that smut resistance is dominant and susceptibility recessive and that segregation for smut resistance has occurred on the basis of a 3:1 ratio.

The only exception to the expected in the $F_2$ behavior of the Markton × Black Mesdag cross, a combination involving the two most highly resistant varieties known, is the occurrence of 1 smutted plant in 69 tested with *Ustilago avenae*-Missouri.

Only 3 of 69 $F_2$ plants of the Markton × Iogold cross were infected with *Ustilago avenae*-Missouri, although 30.1 percent of the Iogold plants, the susceptible parent, were smutted. No infection whatever was obtained with *U. avenae*-Fulghum, as was to be expected. An inconsistent result was obtained with *U. levis*-Missouri in that each parent showed some slight infection, whereas the $F_2$ plants were entirely free from smut.

TABLE 1.—*Reaction to 3 races of smut of inoculated $F_2$ plants of 6 oat crosses and their parental varieties, grown at the Brooklyn Botanic Garden, Brooklyn, N.Y., 1928 and 1929*

| Parental variety or cross | Hybrid no. assigned by— | | Plants inoculated with— | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Division of Cereal Crops and Diseases | Brooklyn Botanic Garden | *Ustilago avenae*-Missouri | | | *Ustilago avenae*-Fulghum | | | *Ustilago levis*-Missouri | | |
| | | | Plants grown | Infected | | Plants grown | Infected | | Plants grown | Infected | |
| | | | *No.* | *No.* | *Pct.* | *No.* | *No.* | *Pct.* | *No.* | *No.* | *Pct.* |
| Monarch Selection (936)[a] | | | 95 | 89 | 93.7 | 30 | 15 | 50.0 | 92 | 0 | 0.0 |
| Monarch Selection (936) × Black Mesdag (926) $F_2$ | 275–1 | 37 | 28 | 5 | 17.9 | 28 | 6 | 21.4 | 72 | 0 | .0 |
| | 275–2 | 38 | 45 | 17 | 37.8 | 27 | 3 | 11.1 | 49 | 0 | .0 |
| Black Mesdag (926) | | | 117 | 0 | .0 | 25 | 0 | .0 | 124 | 0 | .0 |
| Black Mesdag (927) | | | 141 | 0 | .0 | 28 | 0 | .0 | 146 | 0 | .0 |
| Markton (934) × Black Mesdag (927) $F_2$ | 2753–1 | 46 | 27 | 1 | 3.7 | 30 | 0 | .0 | 79 | 0 | .0 |
| | 2753–2 | 47 | 42 | 0 | .0 | 55 | 0 | .0 | 44 | 0 | .0 |
| Markton (934) | | | 152 | 0 | .0 | 25 | 0 | .0 | 148 | 0 | .0 |
| Markton (933) | | | 154 | 0 | .0 | 16 | 0 | .0 | 153 | 1 | .7 |
| Markton (933) × Iogold (930) $F_2$ | 2737–1 | 43 | 25 | 1 | 4.0 | 25 | 0 | .0 | 78 | 0 | .0 |
| | 2737–2 | 44 | 44 | 2 | 4.5 | 26 | 0 | .0 | 57 | 0 | .0 |
| Iogold (930) | | | 153 | 46 | 30.1 | 26 | 0 | .0 | 147 | 6 | 4.1 |
| Cornellian (928) | | | 169 | 90 | 53.3 | 23 | 0 | .0 | 172 | 0 | .0 |
| Cornellian (928) × Markton (935) $F_2$ | 2754–1 | 48 | 27 | 2 | 7.4 | 20 | 0 | .0 | 70 | 0 | .0 |
| | 2754–2 | 49 | 40 | 1 | 10.0 | 27 | 0 | .0 | 45 | 2 | 4.4 |
| Markton (935) | | | 175 | 0 | .0 | 26 | 0 | .0 | 173 | 0 | .0 |
| Markton (932) | | | 183 | 0 | .0 | 29 | 0 | .0 | 151 | 0 | .0 |
| Richland (938) × Markton (932) $F_2$ | 2712–1 | 41 | 28 | 0 | .0 | 28 | 0 | .0 | 78 | 2 | 2.6 |
| | 2712–2 | 42 | 39 | 1 | 2.6 | 26 | 0 | .0 | 49 | 3 | 6.1 |
| Richland (938) | | | 110 | 9 | 8.2 | 22 | 0 | .0 | 109 | 12 | 11.0 |
| Richland (937) | | | 135 | 6 | 4.4 | 119 | 1 | .8 | 13 | 9 | 69.2 |
| Richland (937) × Fulghum (929) $F_2$ | 2710–1 | 39 | 48 | 16 | 33.3 | 51 | 16 | 31.4 | 21 | 7 | 33.3 |
| | 2710–2 | 40 | 26 | 9 | 34.6 | 66 | 23 | 34.8 | 27 | 10 | 37.0 |
| Fulghum (929) | | | 159 | 6 | 3.8 | 113 | 89 | 78.8 | 24 | 2 | 8.3 |

[a] Numbers in parentheses indicate special seed numbers assigned to strains of these varieties inoculated and observed by G. M. Reed.

Only 6 of 67 $F_2$ plants of the Cornellian × Markton cross were infected with *Ustilago avenae*-Missouri, yet the susceptible parent variety showed an average infection of 53.3 percent, there being no infection whatever in the Markton parent. Neither of these parent varieties reacted to *U. levis*-Missouri, but 2 of the $F_2$ plants were infected with this smut.

The reaction of the $F_2$ plants of the Richland × Markton cross to the races of smut corresponded rather closely to that of the two parental varieties.

In the case of each of the three races of smut, approximately one-third of the inoculated $F_2$ plants of the Richland × Fulghum cross were infected, and no marked difference was obtained in the behavior of the two hybrids. However, it will be seen from table 1 that an approximate average of 34 percent of infection was obtained with *U. avenae*-Missouri, yet the parents, Richland and Fulghum, showed only 4.4 and 3.8 percent of infection, respectively.

### $F_3$ PROGENIES

As previously stated, two sets of seed were usually taken from each $F_2$ plant, one set being inoculated with *Ustilago avenae*-Missouri, and the other with *U. levis*-Missouri. With the exception of the Richland × Fulghum cross, no $F_3$ progenies inoculated with *U. avenae*-Fulghum were grown. On the basis of the treatment of the $F_2$ plants three groups of $F_3$ progenies were available for study: (1) Progenies descended from $F_2$ plants inoculated with *U. avenae*-Missouri, (2) progenies descended from $F_2$ plants inoculated with *U. avenae*-Fulghum, and (3) progenies descended from $F_2$ plants inoculated with *U. levis*-Missouri.

Table 2 presents the results obtained in $F_3$ after inoculation with all races of smut. For convenience and comparison, the $F_3$ progenies are grouped into three main classes: (1) Resistant, in which no infected plants were observed; (2) segregating, in which from 1 to 50 percent of the plants were infected; and (3) susceptible, which contained more than 50 percent of smutted individuals. The percentages of infection in the inoculated parental varieties and in the $F_2$ plants and $F_3$ progenies are shown.

### Reaction to Ustilago avenae-Missouri

As shown in table 2, the reaction of the $F_3$ progenies of hybrids 37 and 38 of Monarch Selection (very susceptible to *Ustilago avenae*-Missouri) × Black Mesdag (resistant to *U. avenae*-Missouri) is in accord with genetic expectation. The results obtained from this cross in $F_3$ confirm the interpretation indicated in $F_2$ that smut resistance is dominant and susceptibility recessive, and that segregation for the inheritance of smut resistance has occurred on the basis of a 3:1 ratio. On the basis of the data obtained it might be expected that there would be no susceptible progenies in the first group of $F_3$ progenies descended from $F_2$ plants tested with *U. avenae*-Missouri, since the $F_2$ parent plants susceptible to *U. avenae*-Missouri should have been eliminated. It is possible, however, that the 4 highly susceptible progenies were descendants from susceptible $F_2$ plants that had escaped infection. Assuming that this is the correct explanation, the remaining 45 progenies of this group show a fairly close approximation of a ratio of 1:2, 16 progenies being resistant and 29 segregating.

TABLE 2.—*Reaction to 3 races of smut of inoculated $F_3$ progenies of 6 oat crosses and their parental varieties, grown at the Brooklyn Botanic Garden, Brooklyn, N.Y., 1929, 1930, and 1931*

| Smut species and races used in inoculation | Infection in parent varieties | | Infection in $F_2$ plants | $F_3$ progenies inoculated with— | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *Ustilago avenae*-Missouri | | | | *Ustilago levis*-Missouri | | | | *Ustilago avenae*-Fulghum | | | |
| | ♀ | ♂ | | Resistant | Segregating | Susceptible | Infected | Resistant | Segregating | Susceptible | Infected | Resistant | Segregating | Susceptible | Infected |
| | *Percent* | *Percent* | *Percent* | *Number* | *Number* | *Number* | *Percent* | *Number* | *Number* | *Number* | *Percent* | *Number* | *Number* | *Number* | *Percent* |
| **MONARCH SELECTION × BLACK MESDAG (HYBRIDS 37–38)** | | | | | | | | | | | | | | | |
| *U. avenae*-Missouri | 93.7 | 0.0 | 30.1 | 16 | 29 | 4 | 67.3 | 29 | 19 | 0 | 39.6 | | | | |
| *U. avenae*-Fulghum | 50.0 | .0 | 16.4 | 17 | 26 | 3 | 63.0 | 28 | 17 | 0 | 37.8 | | | | |
| *U. levis*-Missouri | .0 | .0 | .0 | 32 | 60 | 28 | 73.3 | 71 | 39 | 1 | 36.0 | | | | |
| **MARKTON × BLACK MESDAG (HYBRIDS 46–47)** | | | | | | | | | | | | | | | |
| *U. avenae*-Missouri | 0.0 | 0.0 | 1.4 | 43 | 11 | 0 | 20.4 | 33 | 4 | 0 | 10.8 | | | | |
| *U. avenae*-Fulghum | .0 | .0 | .0 | 30 | 9 | 1 | 25.0 | 31 | 9 | 0 | 22.5 | | | | |
| *U. levis*-Missouri | .0 | .0 | .0 | 60 | 18 | 0 | 23.1 | 64 | 12 | 0 | 15.8 | | | | |
| **MARKTON × IOGOLD (HYBRIDS 43–44)** | | | | | | | | | | | | | | | |
| *U. avenae*-Missouri | 0.0 | 30.1 | 4.3 | 36 | 20 | 0 | 35.7 | 34 | 3 | 0 | 8.1 | | | | |
| *U. avenae*-Fulghum | .0 | .0 | .0 | 28 | 12 | 0 | 30.0 | 39 | 1 | 0 | 2.5 | | | | |
| *U. levis*-Missouri | .7 | 4.1 | .0 | 49 | 29 | 0 | 37.2 | 68 | 7 | 0 | 9.3 | | | | |
| **CORNELLIAN × MARKTON (HYBRIDS 48–49)** | | | | | | | | | | | | | | | |
| *U. avenae*-Missouri | 53.3 | 0.0 | 9.0 | 19 | 21 | 1 | 53.7 | 23 | 5 | 0 | 17.9 | | | | |
| *U. avenae*-Fulghum | .0 | .0 | .0 | 24 | 13 | 2 | 38.5 | 32 | 6 | 0 | 15.8 | | | | |
| *U. levis*-Missouri | .0 | .0 | 1.7 | 43 | 29 | 1 | 41.1 | 41 | 13 | 0 | 24.1 | | | | |
| **RICHLAND × MARKTON (HYBRIDS 41–42)** | | | | | | | | | | | | | | | |
| *U. avenae*-Missouri | 8.2 | 0.0 | 1.5 | 9 | 13 | 0 | 59.1 | 7 | 4 | 0 | 36.4 | | | | |
| *U. avenae*-Fulghum | .0 | .0 | .0 | 18 | 8 | 1 | 33.3 | 19 | 5 | 0 | 20.8 | | | | |
| *U. levis*-Missouri | 11.0 | .0 | 3.8 | 45 | 17 | 0 | 27.4 | 44 | 15 | 0 | 25.4 | | | | |
| **RICHLAND × FULGHUM (HYBRIDS 39–40)** | | | | | | | | | | | | | | | |
| *U. avenae*-Missouri | 4.4 | 3.8 | 33.8 | 7 | 20 | 2 | 75.9 | | | | | 0 | 23 | 2 | 100.0 |
| *U. avenae*-Fulghum | .8 | 78.8 | 33.3 | 18 | 39 | 8 | 72.3 | | | | | 8 | 19 | 0 | 70.4 |
| *U. levis*-Missouri | 69.2 | 8.3 | 35.4 | 5 | 14 | 1 | 75.0 | | | | | 4 | 16 | 0 | 80.0 |

The results obtained in the second group of $F_3$ progenies (descended from $F_2$ plants tested with *Ustilago avenae*-Fulghum) of Monarch Selection × Black Mesdag indicate some correspondence in the reaction of the hybrids to the two races of loose smut used in these experiments. If the reaction of the different progenies to these two smuts is not correlated, a much larger number of susceptible families in the group inoculated with *U. avenae*-Missouri would be expected. It is unfortunate that no $F_3$ data are available from progenies of plant material that had been inoculated in $F_2$ with *U. avenae*-Fulghum. Such data might indicate the extent of the similarity in the behavior of the hybrids toward the two smuts.

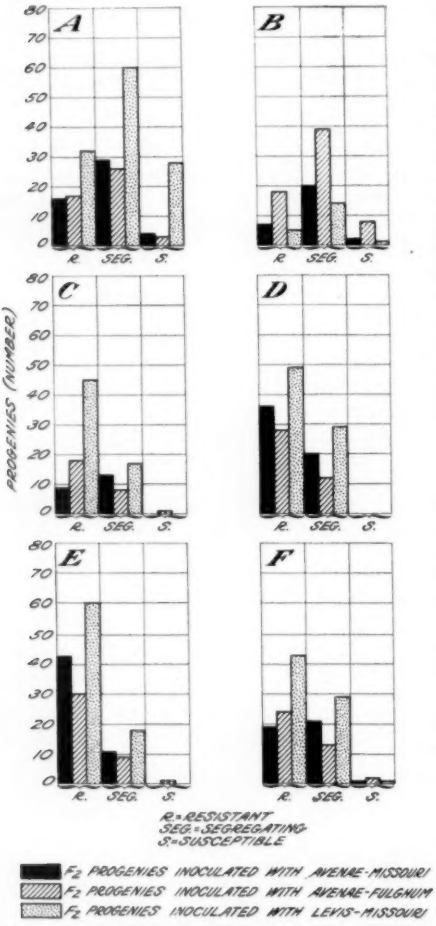


FIGURE 1—Reaction to *Ustilago avenae*-Missouri of $F_3$ progenies for each of six oat crosses, grown from $F_2$ plant populations that had been inoculated with *U. avenae*-Missouri, *U. avenae*-Fulghum, and *U. levis*-Missouri: *A*, Hybrids 37 and 38; *B*, hybrids 39 and 40; *C*, hybrids 41 and 42; *D*, hybrids 43 and 44; *E*, hybrids 46 and 47; *F*, hybrids 48 and 49.

The 120 $F_3$ progenies of the third group of $F_3$ progenies descended from $F_2$ plants tested with *Ustilago levis*-Missouri of Monarch Selection × Black Mesdag showed a ratio of approximately 1 resistant to 2 segregating to 1 susceptible. No $F_2$ plants had been eliminated, and consequently $F_3$ progenies of the different classes might be expected. Of the 28 susceptible progenies obtained, 1 showed 64.2 percent of infection, 2 showed from 70.1 to 80 percent, 5 from 80.1 to 90 percent, and 20 from 90.1 to 100 percent.

The occurrence of smut in the $F_3$ progenies of hybrids 46 and 47 (Marketon × Black Mesdag) was unexpected. As shown in table 2, a large number of the progenies were resistant. However, approximately one-fourth of the progenies contained smutted individuals. One showed as much as 56.2 percent of infection, an interesting result which is difficult of explanation. The behavior of this cross is treated more fully on page 1081.

In $F_3$ progenies of hybrids 43 and 44 (Markton × Iogold) there were a large number of resistant progenies in each $F_2$ smut group, but no progeny was classified as susceptible.

Hybrids 48 and 49 (Cornellian × Markton) involve a cross between a resistant variety and one that is rather susceptible, the smut in the Cornellian plants ranging from 27.2 to 72 percent in the different tests. As shown in table 2, a predominance of resistant progenies was obtained in the $F_3$. Furthermore, only four progenies were classified as susceptible, and the highest percentage of infection obtained in any of these was less than 70. The data for the $F_2$ and the $F_3$ generations do not indicate any simple dominance and segregation.

Hybrids 41 and 42 (Richland × Markton) involve a cross between a resistant variety and one that shows a very low percentage of infection. In the $F_2$ generation 67 plants were inoculated and only 1 was infected. Only 1 $F_3$ progeny was classified as susceptible, 70.5 percent of the individuals being infected. Most of the progenies were entirely resistant.

The Richland and Fulghum parents of hybrids 39 and 40 were slightly susceptible to *Ustilago avenae*-Missouri, both showing less than 5 percent of infection. In contrast to the two parental varieties, a comparatively large number of the $F_2$ hybrids were infected. In each of the three groups of $F_3$ progenies there were 2 or 3 times as many segregating progenies as there were resistant ones. Susceptible progenies also were found in each group, most of the groups containing a high proportion of infected individuals.

The number of infected $F_3$ progenies in each of the six crosses inoculated with *Ustilago avenae*-Missouri is shown in figure 1.

### Reaction to Ustilago levis-Missouri

As has already been shown, the $F_2$ generations of the six crosses were inoculated and tested with *Ustilago levis*-Missouri, and $F_3$ progenies of five of the six crosses were tested with the same race of smut. Table 2 shows that no experiments with *U. levis*-Missouri were conducted with $F_3$ progenies of hybrids 39 and 40 (Richland × Fulghum).

Many $F_3$ progenies in the *Ustilago levis*-Missouri series contained smutted individuals, although both parental varieties of the crosses were resistant (table 2). Hybrids 37 and 38 (Monarch Selection × Black Mesdag) included 128 resistant $F_3$ progenies, 75 segregating, and 1 that was classed as susceptible. This susceptible progeny contained 22 plants, of which 13 (59 percent) were smutted. The 75 segregating progenies contained 1,492 plants, of which 205 were smutted. Hybrids 46 and 47 (Markton × Black Mesdag) included 128 resistant progenies, 25 segregating, and none that was susceptible; the 25 segregating progenies included 493 plants, of which 50 were infected. Hybrids 48 and 49 (Cornellian × Markton) included 96 resistant progenies, 24 segregating, and none that was susceptible; the 24 segregating progenies included 479 plants, of which 43 were infected. In nearly all these segregating progenies the number of infected plants was small—usually but 1 or 2 being observed.

The number of infected $F_3$ progenies in each of five crosses inoculated with *Ustilago levis*-Missouri is shown in figure 2.

### Reaction to Ustilago avenae-Fulghum

Studies were made with the Fulghum race of *Ustilago avenae* only on the $F_3$ plants of hybrids 39 and 40 (Richland × Fulghum). In the various experiments, Fulghum showed 78.7 percent of infection with this race of smut, whereas Richland was nearly free from infection. No resistant $F_3$ progeny was obtained in the first group (descended from $F_2$ plants tested with *Ustilago avenae*-Missouri). Very few susceptible $F_3$ progenies were obtained in any of the groups; several might have been expected in the first and third groups, unless there was some correspondence in the inheritance of resistance to all three races of smut. Comparatively high susceptibility in the $F_2$ generation to all three races of smut was associated with comparatively few $F_3$ progenies susceptible to *U. avenae*-Fulghum.

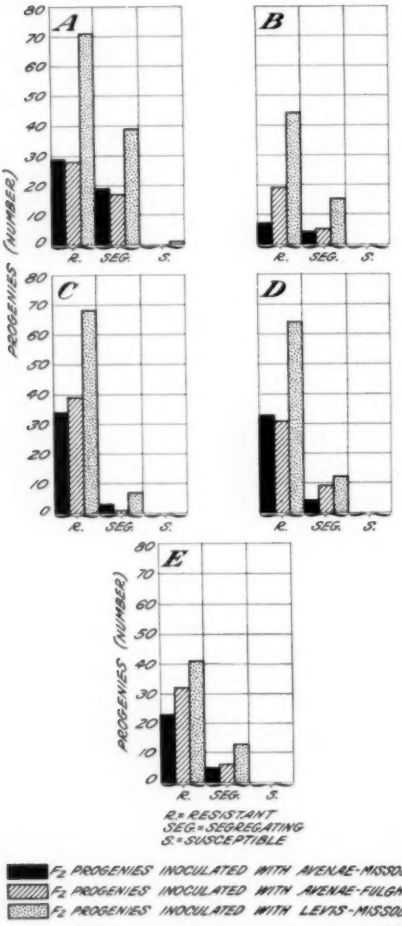


FIGURE 2.—Reaction to *Ustilago levis*-Missouri of $F_3$ progenies for each of five oat crosses, grown from $F_2$ plant populations that had been inoculated with *U. avenae*-Missouri, *U. avenae*-Fulghum, and *U. levis*-Missouri: *A*, Hybrids 37 and 38; *B*, hybrids 41 and 42; *C*, hybrids 43 and 44; *D*, hybrids 46 and 47; *E*, hybrids 48 and 49.

## DISCUSSION

The results obtained from inoculating $F_3$ plants of hybrids 37 and 38 (Monarch Selection × Black Mesdag) with *Ustilago avenae*-Missouri show a simple dominance for resistance to this smut. These results are in accord with those reported by Reed (*8, 10, 12, 13*) in his studies on smut resistance in hybrids of Hull-less × Black Mesdag, Early Gothland × Victor, and Early Gothland × Monarch. In these crosses one parent was susceptible to the particular races of smut used and the other was resistant. The results showed a simple dominance for the inheritance of resistance, segregation occurring on the basis of a 3:1 ratio in $F_2$. The results obtained in $F_3$ were in harmony with the interpretation.

The presence of segregating progenies in $F_3$ in certain crosses in which no infection was expected is doubtless connected with the behavior of certain oat varieties. Occasionally an infected plant is found in a row of Markton when this variety is inoculated with *Ustilago levis*-Missouri. Fulghum frequently shows a small percentage of infection with both *U. avenae*-Missouri and *U. levis*-Missouri. Iogold and Cornellian show a comparatively high percentage of smutted plants. It has, however, been very difficult to approach 100 percent of infection in either of these varieties.

The further question arises as to the extent to which the fungus develops in individuals of such varieties as Markton, Fulghum, and Iogold, the plants of which finally appear normal. Kolk (*5*) has shown that, in the resistant Black Mesdag, *Ustilago avenae*-Missouri penetrates and ramifies more or less extensively throughout the coleoptile. Apparently, however, it does not gain entrance into the developing embryonic tissue.

Bayles and Coffman (*1*), in studies of the effect on germination of removing the hulls from seed of certain oat varieties, report a reduction of 5.1 percent in seedling emergence in the Markton variety when seed with hulls removed was inoculated with smut. A similar reduction was observed in the susceptible varieties Early Champion and Sixty-Day. Stanton et al. (*18*), reporting studies on the effect of removing the hull on seedling emergence in oat varieties, showed that although Markton gave no apparent indication of smut infection, yet seedling emergence was reduced by 2.9 percent through inoculation with smut. It was concluded from this evidence that Markton is not completely free from infection by the smut organism.

The occurrence of marked smut infection in $F_3$ progenies of the Markton × Black Mesdag cross is difficult to explain. It is possible that complementary factors may account for the reduction, or apparent breaking down, of resistance in the $F_3$ progenies of this cross. Briggs (*2*) has observed the occurrence of smutted plants in some of his resistant progenies of hybrids between Hard Federation and Hussar wheat varieties. No smut appeared in the $F_2$ and $F_3$, but in the $F_4$ and subsequent generations slightly susceptible families were obtained. Briggs interprets his results on the basis of modifying factors.

In the Markton × Black Mesdag cross, 25 percent of the $F_3$ progenies descended from $F_2$ plants inoculated with *Ustilago avenae*-Fulghum were infected (table 2). The occurrence of smut infection in so many progenies is hardly analogous to the appearance of a few smutted individuals in $F_4$ and subsequent generations of certain wheat crosses reported by Briggs (*2*). As a consequence, these results cannot be interpreted on the basis of modifying factors. It is probable that complementary factors may offer a more satisfactory basis for interpretation, since so high a percentage of the $F_3$ progenies were smutted. Both Markton and Black Mesdag may carry complementary factors for susceptibility, which, when brought together through hybridization, may produce smutted plants.

Another interesting result is the relation between the number of resistant, segregating, and susceptible $F_3$ progenies. In the *Ustilago levis*-Missouri series there is a preponderance of resistant progenies in all five hybrids. The same is true in the *U. avenae*-Missouri series of

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hybrids 41 and 42, 43 and 44, 46 and 47, and 48 and 49. Of course these results would be expected to a large extent on the basis of the behavior of the parental varieties, since none of them showed anything like 100 percent of infection.

The loss of the susceptible individuals in these hybrids introduces a difficulty in the complete analysis of smut inheritance. The smutted plants being destroyed, their subsequent generations cannot be grown.

## SUMMARY

This paper presents results obtained with six oat hybrids, involving crosses between varieties that differ in their behavior to certain races of the oat smuts.

$F_2$ plants of all these crosses, inoculated with *Ustilago avenae*-Missouri, *U. levis*-Missouri, and *U. avenae*-Fulghum, were grown and studied. Progenies of all 6 crosses inoculated with *U. avenae*-Missouri, progenies of 5 crosses inoculated with *U. levis*-Missouri, and progenies of 1 cross inoculated with *U. avenae*-Fulghum also were grown in $F_3$, and their reaction to these smuts was recorded.

The data for hybrids 37 and 38 (Monarch Selection × Black Mesdag) inoculated with *Ustilago avenae*-Missouri indicate that smut resistance is inherited on the basis of a 3:1 ratio.

A most noteworthy feature is the occurrence of considerable smut in some $F_3$ progenies of hybrids descended from entirely resistant parents.

In most of the hybrids completely resistant progenies predominated over segregating progenies; there were very few susceptible progenies recorded.

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# TESTING ALFALFA FOR RESISTANCE TO BACTERIAL WILT[1]

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## INTRODUCTION

In 1930 the writer (*3*)[2] described a procedure whereby it seemed possible to compare alfalfa (*Medicago sativa* L.) varieties in regard to their resistance to bacterial wilt, caused by *Phytomonas insidiosa* McCulloch, in the space of a single year, thereby avoiding the delay and climatic hazards inevitable in a field test in infested soil. The procedure described permitted not only the comparison of the degree of resistance in varieties but also the selection of resistant plants from which resistant strains may be developed. During 3 years the writer has used this method in comparing resistance in plants from a considerable number of seed lots of alfalfa. Modifications of the procedure, which would permit testing larger numbers of plants with the facilities available for the purpose at Madison, Wis., have been tested. The development of the parasitic bacteria in resistant and in susceptible plants has been compared. The present paper is a report on the progress of the work thus outlined.

In 1930 Peltier and Tysdal (*7*) published results from a single but extensive trial of the method described by the writer (*3*). In 1933 Peltier (*5*) published additional data of a similar character. All of these results are substantially in agreement with those presented here. In 1932 Peltier and Schroeder (*6*) in a study of the nature of resistance of alfalfa to wilt reached the conclusion that in the material studied resistance was in the main associated with morphological features of the root. The writer is unable to corroborate this conclusion. These results by Peltier and his associates will be discussed later in this paper.

## COMPARISON OF VARIETIES FOR RESISTANCE

The procedure in comparing seed lots for resistance has been little altered from that originally suggested (*3*). The seed is planted in flats in the greenhouse, preferably in November, but when necessary as late as the middle of January. When the seedlings are about a month old they are transplanted, usually into beds where they are spaced about 1½ inches apart in rows 3 inches apart. As early in May as possible the seedlings are washed from the soil, inoculated, and transplanted to the field, where they are spaced 4 inches apart in rows 10 inches apart. In 1930, inoculation was made by scraping opposite sides of the root lightly with a knife under a bacterial suspension from several pure cultures of different origin. In the 2 following years incision was made through the bark with a sharp knife under a bacterial suspension instead of scraping. A second inoculation was

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[2] Reference is made by number (italic) to Literature Cited, p. 1098.

made of the plants in the entire plot in 1930 and of a part of those in the plot in 1931 in early August in the following manner. After all plants showing disease symptoms were pulled, earth was removed from around the crowns of those remaining, and a thin slice of diseased tissue from one of the infected plants was inserted in an incision through the bark of the taproot. The earth was then replaced about the crowns and the ground was well watered. This second inoculation cannot be made rapidly, and therefore it was not carried out with plants from the seed lots from Turkistan in 1931 nor was it attempted at all in 1932. Three cuttings have usually been made in the early bloom stage.

After active growth had ceased in the fall, the plants were dug for examination and divided into two classes, namely, those infected and those uninfected. Evidence of infection was first sought by cutting the main root of each plant at about 6 inches below the crown. If no discoloration characteristic of the disease could be found in any root at this distance from the crown, the scar of the wound made at inoculation was examined. If no discoloration was found here after making such incision as seemed necessary, the plant was classed as uninfected. Such an examination will not always reveal local infections near the crown, and therefore occasional plants judged uninfected and placed in the greenhouse have subsequently developed disease; but these occasional errors in classification are preferable to a more thorough examination of the plant which destroys its immediate usefulness in seed production.

The method outlined may be varied by starting the plants at different times in the year and inoculating at different stages of development in the greenhouse as well as in the field. Several variations which might be serviceable if successful have been tried. Chief among these are the following:

(1) Seed sown in the field in early spring.
  (*a*) Inoculation at transplanting to rows in the field, as with greenhouse-grown plants, as soon as seedlings are large enough to inoculate conveniently through wounds in the roots.
  (*b*) Inoculation at transplanting to the greenhouse in which the plants are grown through the summer.
  (*c*) Inoculation of spaced plants without transplanting.
(2) Seed sown in the field in late July or August; inoculation at transplanting to the greenhouse in October.

The first variation was tried extensively in 1930. Owing in part to a thick stand, the plants did not attain sufficient size for transplanting until late June, and transplanting extended into early July, when the temperature was high. While water was freely used at transplanting and subsequently, the plants did not make rapid growth during the entire summer, though few died. Infection was less than by the routine method, especially in the more resistant class. The period from inoculation to the end of the summer appeared much too short for advanced development of the disease in infected plants. The procedure gave useful comparisons between seed lots, but it did not result in the infection of nearly so many plants as did the routine method. It has not been tried further.

The second variation was likewise tried in 1930 in the hope of extending the growing season somewhat and thus obtaining a more thorough elimination of the susceptible plants. Seemingly excellent conditions for infection were provided by shading the greenhouse so

that the temperature did not rise above 30° C. and by keeping the soil well watered. Nevertheless, infection was even less than in plants transplanted to the field at the time the greenhouse experiment was begun. The method appeared useless and was not tried further. In general, field-grown seedlings transplanted at any other time than in late autumn make less rapid recovery and subsequent growth than greenhouse-grown seedlings.

The inoculation of spaced seedlings grown in the field by digging around the plants and applying a bacterial suspension to a wound made in the root or by inserting bits of diseased tissue in the root has invariably proved a highly effective method in securing infection. In 1931 it was tried extensively. The disadvantages of this method are the amount of labor involved and the fact that in this latitude seedlings from the spring sowing do not attain sufficient size for inoculation until the growing season is far advanced. Thus when winter comes the disease has not progressed far, especially in the more resistant plants. It should be noted that inoculation by fragments of diseased tissue dipped in bacterial suspension and inserted in slits in the bark of the roots has given slightly higher infection than any other method tried. Tables of the results of this method as compared with results obtained by the use of several pure cultures are omitted for the sake of brevity.

Where seedlings grown in the field in late summer and early autumn were inoculated upon being transplanted to the greenhouse, it seemed that inoculation would be made into the most susceptible tissue the plant would produce under field conditions and that in consequence disease development would be rapid. In a single trial made with about 900 plants from 12 seed samples the anticipated high infection was obtained, an infection approximately equal to that obtained in the field from the inoculation of greenhouse seedlings. The method has not been used further because it requires much greenhouse space.

TABLE 1.—*Comparative test of alfalfa varieties and seed lots for resistance to bacterial wilt, 1930*

[Inoculation was made with pure culture at transplanting in May and by use of diseased tissue in August]

| Origin or variety | Seed lot no.[a] | Plants inoculated | | Origin or variety | Seed lot no.[a] | Plants inoculated | |
|---|---|---|---|---|---|---|---|
| | | Total | Uninfected | | | Total | Uninfected |
| | | *Number* | *Percent* | | | *Number* | *Percent* |
| Arizona | 1289 | 217 | 1.5 | Montana | 1300 | 218 | 2 |
| Argentine | 1332 | 201 | 1.5 | Peruvian | 1290 | 219 | 0 |
| Baltic | 597 | 181 | 1 | Ladak | 1334 | 233 | 30 |
| Cossack | 1260 | 145 | 1.6 | South Dakota | 875 | 216 | 4 |
| Grimm | 999 | 437 | 1 | Do | 1173 | 228 | 2 |
| Do | 1335 | 195 | 1 | Turkistan | 1356 | 149 | 24 |
| Kansas (Wichita) | 1224 | 302 | 0 | Do | 84371 [b] P.I. | 118 | 44 |
| Kansas (Derby) | 1225 | 209 | 9.5 | | | | |
| Do | 1301 | 166 | 5 | Utah | 1157 | 158 | 3 |
| Do | 1302 | 194 | 1 | Do | 1219 | 134 | 0 |
| Do | 1303 | 202 | 2.5 | Do | 1245 | 225 | .5 |
| Do | 1304 | 253 | 3 | | | | |

[a] Identification number used at the Wisconsin Experiment Station.
[b] Identification number of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, U.S. Department of Agriculture.

From this survey of possible variations in the methods for determining resistance in 1 year, it appears that the schedule originally suggested best combines convenience and effectiveness. The first extended comparison of seed lots of alfalfa by this routine method for determining wilt resistance was made in 1930. The seed samples used were largely American regional strains contributed by Prof. L. F. Graber, of the Wisconsin Agricultural Experiment Station. The results of the 1930 trials are presented in table 1. Very few plants from the American strains remained uninfected, whereas the two seed samples from Turkistan and the one from Ladak were strikingly distinguished by the comparatively large number of uninfected plants.

In 1931 some of the seed samples collected by H. L. Westover in Turkistan in 1929 and in Spain and northern Africa in 1930, together with a few miscellaneous samples of diverse origin, were tested. The plant populations from seed of undoubted Turkistan origin were sharply distinguished from all others by greater resistance. The results are summarized in table 2. A few additional samples from the same regions were compared in 1932 with the same result. This outcome appears to accord with the very extensive results obtained by Peltier and Tysdal (*7*) and by Peltier (*5*). The percentage of uninfected plants in the results cited is usually higher than that shown here. Such difference in the susceptible varieties may be due to the fact that in this class of plants a second inoculation was made by the writer, whereas in obtaining the results cited, but a single inoculation was made.

TABLE 2.—*Resistance to bacterial wilt in alfalfa from foreign sources after artificial inoculations in 1931–32*

| Source | Seed lots | Plants inoculated | | | |
|---|---|---|---|---|---|
| | | Number | Percentage uninfected | | |
| | | | Highest | Lowest | Average |
| Turkistan: | *Number* | | *Percent* | *Percent* | *Percent* |
| Samarkand | 4 | 577 | 80 | 33 | 55 |
| Tashkent | 11 | 1,926 | 80 | 23 | 50 |
| Ferghana | 2 | 291 | 52 | 43 | 47 |
| Khiva | 1 | 59 | | | 54 |
| Bukhara | 1 | 108 | | | 69 |
| Manchuria | 1 | 63 | | | 0 |
| China | 4 | 390 | | | .3 |
| Spain | 45 | 4,873 | 1 | 0 | .3 |
| Portugal | 4 | 500 | 1 | 0 | .2 |
| Northern Africa | 33 | 4,203 | 1 | 0 | .5 |
| South America | 4 | 491 | | | .2 |

Alfalfa, then, falls into two distinct major classes with respect to resistance: A resistant class consisting of or derived more or less directly from Turkistan or Ladak, and a susceptible class comprising alfalfa from all other sources tested thus far. The result may be stated in another way. Resistance to bacterial wilt appears to be a characteristic of alfalfa in the region, or at least in much of the region, credited as being its place of origin. The boundaries of the region in which high resistance is a uniform characteristic are not precisely delimited by the data at hand. In the dissemination of the plant from this region of origin and adaptation to other regions

where it is now grown the resistant character appears to have been largely lost, whatever habit of growth the plant has assumed in its new environment. That the disease occurs in Turkistan is attested by the fact noted previously,[3] that the bacteria causing it were recovered from two plants collected by Westover at a small village north of Bukhara. However, it is unlikely that the disease is abundant or conspicuous in this region at present, as Westover has stated to the writer that he recognized the disease only at this location, in a field that had been excessively watered. Thus it appears possible that the parasite has been constantly associated with the plant in Turkistan, enforcing a selection for resistance in that region, and that the parasite has not followed the plant elsewhere to continue that selection until a comparatively recent introduction into the United States; but facts are not at hand whereby this suggestion may be confirmed.

## COMPARISON OF INDIVIDUAL PLANTS FOR RESISTANCE

Whatever the historical background of the present regional separation of the resistant and susceptible varieties of alfalfa, the present task is production by artificial selection of resistant varieties suited to regional needs. Two sources of material are available for use: (1) Strains of highly resistant alfalfa from central Asia and (2) individual resistant plants that may be found in present commercial varieties. If individual plants from susceptible varieties are chosen for the building of varieties, much use of artificial inoculation for the elimination of susceptible plants from populations will be required. Therefore, a critical appraisal of the accuracy and limitations of artificial inoculation as a tool both in comparison of varieties and in selection should be made.

That a test carried out in the field where conditions differ from year to year will give identical results is hardly to be expected; and in fact it has not always given closely similar results in the same season from transplantings made but a short space of time apart. Large lots of seedlings have been divided and transplanted at several dates through May and June, but differences in the results of these experiments have not been greater than differences that have occurred from inoculations made a few days apart. In a few Turkistan seed samples differences in the percentages of uninfected plants have amounted to 30 percent in exceptional cases. In susceptible varieties differences have not been so great, due perhaps in part to the second inoculation. Such differences were not only perplexing in the plots at Madison, but became even more so when an attempt was made to duplicate some of the work elsewhere. For instance, in 1930 some of the seed samples numbered in table 1 were grown by C. O. Grandfield at Manhattan, Kans., in the greenhouse, and inoculated at transplanting with the same cultures as at Madison. At Manhattan the percentage of uninfected plants was determined by Grandfield in the autumn. On the whole, the percentage of uninfected plants was greater at Manhattan, probably because they were inoculated but once, but this result was not consistent in all the lots. For instance, one sample gave 25 percent of uninfected plants at Manhattan and but 2.5

[3] JONES, F. R. BACTERIAL WILT OF ALFALFA IN TURKISTAN. U.S. Dept. Agr. Bur. Plant Indus. Plant Disease Rptr. 14: 125. 1930. [Mimeographed.]

percent at Madison, and a sample giving 7 percent of uninfected plants at Madison gave but 2.5 percent at Manhattan.

A study of planting and climatic data has been made in an effort to explain these differences; but thus far no fact or set of facts has been found with which the differences can be correlated. That the method of inoculation is usually sufficiently effective to prevent many plants from escaping infection, even though they escape wounding, was shown in 1931, when groups of 10 plants were set unwounded and uninoculated at intervals in the plot so that difference in growth between diseased and healthy plants might be observed. At the end of the season all these groups of uninoculated plants were found infected almost as severely as the inoculated plants, except in the final transplanting made early in June. Emphasis upon these irregularities in the results of inoculation should not obscure the fact that most repeated trials are closely consistent in result. A second inoculation of plants that have not shown evidence of disease in the foliage by the end of July appears to make results more consistent than does a single inoculation. However, this biological test, carried out in the field under varying climatic conditions, has not been and perhaps cannot be standardized to produce precise comparisons.

Thus far the discussion has been confined to the consistency of the test in defining the resistance of plant populations. In the selection of resistant plants it is even more important to know how accurately the test defines or can be adapted to define the resistance of individual plants. At the conclusion of a test in autumn some plants are found uninfected, others with varying degrees of disease development. The questions arise whether any or all of these uninfected plants are uninfectable, or immune, and whether degrees of disease development represent intrinsic resistance or a more or less accidental retardation in disease development. In an effort to answer these questions, plants showing apparent degrees of disease development were set in the greenhouse for further observation. Most of these plants developed disease and soon died. Thus the tentative conclusion was reached that degree of infection in plants from susceptible varieties, even after two inoculations, is not a reliable index of degree of resistance in those plants, at least, not of a sufficiently high degree of resistance to be of practical importance. In fact, occasional plants among those showing no infection at all have developed disease and died as though very susceptible. These results may indicate that resistance, at least a low degree of resistance, is not stable in growing plants. Thus one of the first and most essential steps in the study of resistance in individual plants consists in the determination of the stability of resistance with reference to the age and environment of the plant, or the description of such degrees of resistance as may be distinguished.

A simple procedure in the determination of the stability of high resistance in plants with reference to age consists in the repeated inoculation of such plants as fail to be infected by the routine method used in the comparison of plants. A considerable number of plants found uninfected in the fall have been reinoculated and set in the ground for growth the following year. In spite of winter protection, such transplanted roots have been killed outright or so badly damaged that no significant results have been obtained. Inoculated populations left in the ground all winter with some protection have been dug

in the spring, and the uninfected plants have been reset in the ground. These have usually failed to grow vigorously after such transplanting; consequently, the reset plants can hardly be regarded as representing the condition of plants that have grown with undisturbed roots. A more promising method of obtaining the desired end consists in the use of cuttings. In the autumn of 1930, cuttings were made from a number of uninfected plants and rooted in the greenhouse. The vigorous plants from these cuttings were inoculated by inserting bits of diseased tissue in the roots and were set in the field in the following spring. A part of the result of this inoculation is given in table 3. Some of the groups of genetically identical plants obtained from cuttings remained free from infection and some groups were infected in part or in all of the individuals. Further trial will be made of these uninfected populations to determine whether infection can be obtained in them; but at present it appears that even from varieties in which 95 percent of the plants are infected in a routine test individuals may be found which are immune to wilt under these summer field trial conditions.

TABLE 3.—*Infection of plants from cuttings the parent plants of which had withstood two inoculations without infection*

| Variety | Plant no. | Diseased | Healthy | Variety | Plant no. | Diseased | Healthy |
|---|---|---|---|---|---|---|---|
| | | *Number* | *Number* | | | *Number* | *Number* |
| Turkistan | 480–1 | 3 | | Montana Common | 1300–1 | | 4 |
| | 480–2 | | 7 | | 1300–3 | | 2 |
| | 480–6 | | 4 | | 1303–2 | | 3 |
| | 480–7 | 4 | | | 1303–3 | | 3 |
| | 480–9 | | 5 | Kansas Common | 1304–1 | 4 | 3 |
| | 480–10 | 2 | 2 | | 1304–2 | 1 | 2 |
| | 2–5 | | 11 | | 1304–4 | 1 | 4 |
| | 2–7 | 4 | 3 | | 1304–5 | | 2 |
| | 1356–5 | 2 | 5 | | 1304–7 | 5 | |
| Grimm | 998–1 | 4 | | | 1304–8 | 2 | 2 |
| Utah Common | 1210–1 | 1 | 1 | | | | |
| | 1158–3 | 4 | 1 | | | | |
| | 1158–4 | 2 | 1 | | | | |

Infection that occurred in this trial was usually slight. No symptoms were observed in the foliage. The disease had progressed very slowly after entering the vascular system. Although the fate of these infected plants in the following year was not determined, it seems probable that in most cases the disease would have been outgrown. On the basis of this opinion, such plants may be placed in a highly resistant class in which infection is possible but in which it tends to remain localized.

A tentative third class of plants, having resistance slightly lower than the preceding yet perhaps enough to be of practical importance if possessed uniformly in a variety, is illustrated by the following example: Three plants of Grimm, two without infection, the third with very slight infection, were selected at the end of the inoculation trial in 1930. Cuttings from these plants were grown in the greenhouse and three from each plant were inoculated in the spring of 1931. At the end of the summer none of these plants showed disease, and they remained in the field through 1932, still vigorous and without evidence of disease in the foliage. When they were dug in the winter, they were found badly infected, invasion extending to the center of the root, and they soon died. Degree of infection and behavior differed little in the nine plants. They were much less

injured by wilt in 2 years than most Grimm plants in a single summer; therefore, they may be regarded as having a degree of resistance somewhat lower than that described in the previous class.

Thus, by means of these field trials of cuttings from individuals, resistant plants may be classified provisionally as immune, highly resistant, and resistant. Such classification is admittedly very crude and unsatisfactory. It may serve to indicate that resistance appears to exist in varying degrees in plants. In rare individuals immunity may be attained, but comparatively high and low degrees of resistance may be distinguished.

## THE NATURE OF RESISTANCE

During the foregoing comparison of varieties with respect to resistance, detailed study has been attempted of the behavior of infection in plants having different degrees of resistance, with a view to describing the differing host-parasite relationship which finds conspicuous expression in these degrees of resistance. In this work methods of approach have been developed and tentative conclusions have been reached which need further confirmation. A discussion of this incomplete work seems warranted, since Peltier and Schroeder (*6*) in a recent publication have reached conclusions radically different from those of the writer.

In the course of the comparative testing of alfalfa seed lots many inoculated plants of resistant or susceptible strains in various stages of disease have been examined and compared. Material has been fixed and stained after being embedded in paraffin, but more frequently living roots have been sectioned with the razor or the sliding microtome. By the latter method discolored areas indicating infection can be examined at the exact point of interest more rapidly and effectively than is possible after the material has been embedded. Sections from living material are fixed in alcohol to hold the bacteria in place and are stained in the usual manner. Thus the relation of the bacteria to large areas of the host tissue may be surveyed in a relatively short time.

Attention was first directed to the initial step in infection, namely, the development of the bacteria in parenchymatous tissue around the wound to which the bacteria were applied. Here it was found that in plants with no vascular invasion at the end of a routine test for resistance, there is usually no invasion of parenchymatous tissue or but a very slight invasion which has not reached the vessels. In plants in which vascular invasion is slight, invasion at the point of inoculation is slight and is not found elsewhere along the invaded vessels. In very susceptible plants invasion of parenchymatous tissue at the point of inoculation is relatively rapid and abundant, and vascular invasion is soon followed by parenchymatous invasion at more or less widely scattered points along the invaded vessels. Occasionally very conspicuous exceptions occur in which extensive invasion of parenchymatous tissue at the point of inoculation is not followed by extensive vascular invasion, but these exceptions appear to form a very small distinct class which need not confuse the discussion here.[4]

[4] In the exceptional plants referred to, the parenchymatous tissue appears to react to invasion by hypertrophy and even by slight hyperplasia. The region of inoculation develops a gall-like swelling. The bacteria usually pass to the very center of the root along the enlarged wood ray cells. Few vessels are nvaded, and these may be scattered to the center of the root. Such plants have been found thus far only n strains of Turkistan alfalfa.

The foregoing results indicate that resistance first finds expression in the failure of the bacteria to establish themselves rapidly and extensively in the parenchymatous tissue of the host, either at the portal of entry, or later from the invaded vessels.

Following the examination of infection in populations presumably more or less heterogeneous, similar comparisons were made with plants of known uniformity, that is, with cuttings from previously tested plants. The supply of such plants has been small, both in quantity and in diversity of origin; therefore, methods were devised whereby it could be used most economically. Chief among these were the inoculation of pieces of roots in an incubator and the reciprocal grafting of plants. The results of the preliminary tests are as follows.

Pieces of roots taken from large plants in the fall were stored under controlled environmental conditions, and infection has been obtained in such pieces as well as in entire plants. The procedure has been used both to test rate of penetration of the bacteria in susceptible plants under different environmental conditions and to compare penetration in susceptible and resistant roots. Results obtained in this way support observations made in growing plants. Very little penetration of the bacteria into the phloem of roots of well-attested resistance has occurred, while abundant penetration of bacteria into roots of susceptible plants has been obtained.

The most spectacular contrast in the development of the bacteria in resistant and susceptible plant tissue has come from inoculation of grafted plants, though this work is as yet meager in quantity. The grafting is easily accomplished between roots of the same diameter, both in the fall with plants from the field and in the spring with greenhouse seedlings ready to set in the field. The usual whip graft has been used, the union tightly bound with twine and covered with melted beeswax. The grafts are packed in moist sphagnum, with a little of the crown exposed, and maintained at a temperature of about 25° C. in a moist chamber for 2 or 3 weeks to hasten the union. The plants are then set in soil with protection from drying until vigorous growth starts. Inoculation has occasionally been made on the cut surfaces at the time of grafting, but usually in the susceptible portion of the graft at the time of planting.

The two portions of the graft appear to maintain essentially their original susceptible or resistant characteristics in this union, although a susceptible top on a resistant root lives longer after infection than it does on its own decaying root. Through the use of grafted plants bacteria can be introduced directly into unwounded and continuously functioning vessels of resistant tissue without having to pass through parenchymatous tissue in which, as previous observation indicated, they were hardly able to penetrate.

A typical contrast of bacterial development in tissues of immune root inoculated in this way with that in a susceptible root is furnished by a fortunate graft in which about 4 cm of immune Turkistan root was inserted in the taproot of a highly susceptible nonhardy plant. This graft was made in December 1931. The lower portion of the susceptible root was inoculated, and the plant grew slowly in the greenhouse during the winter. In the spring it was set in the field, where it grew vigorously, showing no symptoms of disease. When dug in October the taproot was about 2 cm in diameter and the graft

unions were smooth, though visible. The crown and taproot were then split vertically; one half was waxed over the wound and set in the greenhouse, and the other half was prepared for microscopic examination. On December 19 the foliage of the greenhouse plant showed symptoms of wilt and was then examined. Sections prepared at the earlier and later dates from this plant show differences only in degree of disease. The entire root, including the inserted portion, was deeply discolored. The new growth formed since the union was a wide band each of summer and autumn wood sharply distinguished. The lower susceptible portion of the root was severely diseased, with extensive invasion of the fall wood both in vessels and in parenchymatous tissue. The upper susceptible portion was less severely invaded, although bacteria had advanced to some of the outermost vessels. In the immune insert, which was almost as deeply discolored as the rest, the bacteria were found only in a few vessels of the summer wood and not anywhere in parenchymatous tissue. Extensive gum formation occurred in the susceptible portion, but only in the summer wood of the immune insert. The discoloration of the immune portion was due largely to the soluble stain, which may have been produced in the susceptible part. So far as could be discovered, vascular connection through the inserted portion was open, permitting distribution of bacteria through it from the infested vessels at either end, and perhaps the bacteria found there had arrived by this route. Previous trials with ink in grafts had demonstrated open vessels through the unions permitting ink particles to pass, though many vessels had sharp turns conducive to clogging.

In other inoculated grafts of resistant tops on susceptible roots, and vice versa, made at about the same time, similar results have been obtained. Disease has progressed in the usual manner in the susceptible portion. Discoloration extends far into the resistant part of the union, although few vessels in that resistant part show clear evidence of bacterial development in those vessels by the presence of matlike colonies over pits. Thus it appears that immunity and very high resistance are manifest not only by failure of the bacteria to develop in the intercellular spaces of the parenchyma of an inoculated plant, but also by a much less luxuriant growth in the pits of those vessels. In no case have bacteria been found invading parenchymatous tissue in the highly resistant part of grafts, however extensively this has occurred in the susceptible part.

From the writer's previous studies of this disease the conclusion has been drawn that without invasion of parenchymatous tissue vascular invasion does not proceed far; and thus if this apparent resistance of the parenchymatous tissue to invasion continues to be found in all plants which are not infected or but slightly infected by repeated inoculation in the usual routine, the resistant character seems to be manifest chiefly in the parenchymatous phase of invasion. The nature of this resistant character is not apparent. The gathering, testing, and increasing of resistant plant material for comparison is in progress, and a more comprehensive comparison of such material is planned.

A theory of the origin of resistance very different from that just outlined has been stated recently by Peltier and Schroeder (*6, p. 2*).

* * * The usual or normal progress of the bacteria from their entry until the death of the plant ensues has been followed.

In the main it has been found that resistance in some alfalfas is associated with certain morphological features, particularly in the root, which inhibit rapid development and invasion of the vital tissues by the bacteria. These morphological differences in susceptible and resistant sorts are inherent, though not absolute, since any variety or strain of alfalfa is made up of a widely diverse lot of individuals. It is for this reason that not a single variety or strain of alfalfa has been found which is completely resistant.

* * * Thus while resistance in alfalfas to wilt is associated with root structure, it is also true that inhibiting or accelerating the rate of growth of either susceptible or resistant sorts will modify the root structure to such an extent that susceptible sorts will become more resistant or resistant alfalfas more susceptible.

* * * There appears to be no direct evidence in any of our physiological or microchemical studies to show that any internal physiologic function of the plant makes one variety more resistant than another, except insofar as morphological modifications may occur under different environmental conditions.

The morphological characteristics of resistant varieties are stated to be as follows: (1) Vessels of smaller diameter, more angular, with heavier wall thickenings; (2) vessel segments shorter, with more obstructions from remaining vestiges of the septa; (3) vessels arranged in groups, with less contact and more intervening fibers; and (4) less frequent contact of vessels with parenchymatous cells. Peltier and Schroeder illustrate these differences by contrasting photomicrographs, but give no measurements of length or diameter of vessel segments. Measurements made by the writer from their photomicrographs (*6, pl. 7*) showing difference in diameter of vessels in a plant of a resistant and a susceptible variety show that the average outside diameter of vessels in the resistant root is about 23$\mu$ and in the susceptible root about 38$\mu$. The writer has examined sections of many resistant and susceptible plants and has not observed such contrast in vessel diameter. However, in such comparisons, measurements are a safer guide than observation, and therefore, in July 1933, plants of 3 resistant and 4 susceptible varieties under test in the field were sectioned for examination. Median longitudinal sections were stained lightly with thionine, and the length and diameter of vessel segments of at least 10 representative vessels distributed across the diameter of the root were measured. The average of these measurements was found, and finally the average of such measurements from 5 plants. The results are given in table 4. No significant difference appears in either length or diameter of vessel segments between the resistant and the susceptible varieties. In all plants vessel diameter is approximately the same as that shown in the cited illustrations of a susceptible plant (*6, pl. 7, b*).

TABLE 4.—*Comparison of average length and diameter of vessel segments in roots of resistant and susceptible varieties of alfalfa under test for resistance to wilt*

[Measurements made in plants taken from the field, July 19, 1933]

| Resistant variety | Average length | Average diameter | Susceptible variety | Average length | Average diameter |
|---|---|---|---|---|---|
| | $\mu$ | $\mu$ | | $\mu$ | $\mu$ |
| Turkistan | 104 | 32 | Grimm | 84 | 38 |
| Hardistan | 90 | 38 | Cossack | 80 | 38 |
| Ladak | 86 | 36 | Canadian Variegated | 88 | 36 |
| | | | South Dakota Common | 80 | 38 |

The writer has sought among the plants in his selections for evidence of the remaining differences between resistant and susceptible plants described by Peltier and Schroeder and has failed to find any of them. It will be very interesting indeed if these investigators have found an environmental condition in which resistant and susceptible varieties show different internal structures, but such structural differences can hardly be regarded as a cause of resistance when similar contrasts in resistance are shown by the same varieties grown in an environment in which these structural differences do not occur. The structural differences between roots of resistant and susceptible varieties here described appear closely similar to those observed by the writer in plants responding to a short period of illumination with short internodes and with long internodes, respectively, when both are grown in short days.

It should be pointed out that Peltier and Schroeder appear to the writer to have built their morphological theory of resistance upon a conception of the development of the disease in the plant which differs in one important respect from that held by the writer (*2*, *4*). The difference in opinion concerns the route by which the bacteria spread within the plant, and may be stated thus. Both agree that in entering a plant through a wound the bacteria first establish themselves in parenchymatous tissue, whence they pass into vessels. From this point interpretations differ. The writer finds parenchymatous invasion arising along those vessels first invaded and believes that by passing between cells of this tissue the bacteria invade other vessels in the same manner in which the first vessels were penetrated at infection. Peltier and Schroeder do not describe such parenchymatous invasion, progressively increasing from initial infection, but describe the passage of bacteria from vessel to vessel by penetration of the thin wall separating opposed pits of contiguous vessels. Although the writer has recognized that passage of bacteria from vessel to vessel does occasionally take place by the dissolution of cell walls (*4*, *p. 70*, *fig. 18*), he has been unable to find convincing evidence that passage occurs through pores in pits as described by Peltier and Schroeder (*6*, *pp. 7–8*). In fact, the writer is unable to verify the existence of the pores described in the cell membrane. The photomicrographs cited as showing these pores or reticulated condition of the membrane (*6*, *pl. 1*, *B*, *C*.) appear to show only a refractive effect of the lignified thickening of the wall, not pores in which bacteria can or do find lodgment and development. In a recent paper, Bailey (*1*) gives illustrations and a description of vestured pits that appear to identify as vestured pits the pit condition in alfalfa illustrated by Peltier and Schroeder.

It appears probable to the writer that Peltier and Schroeder overlooked the presence of parenchymatous invasion in the early stages of disease development, widely scattered and tenuous as it often is along the invaded vessels. For instance, they state (*6*, *p. 27*):

> As a rule plants in which bacteria are present in the parenchymatous tissues in the fall do not survive the winter or spring because of the small amount of root reserves found in plants in this stage of disease development.

This statement does not accord with the writer's experience. In the spring of 1933 special search was made for local invasions of parenchyma in plants overwintering with but a trace of disease. Among plants brought from an old field, invasions in parenchyma

were demonstrated by staining in 80 percent of the plants examined, and in 90 percent of overwintering plants artificially inoculated the previous summer. Invasion of parenchyma has been found, in the writer's opinion, sufficiently associated with all stages of active disease development to account for progress of bacteria to new vessels.

## RESISTANCE AS INFLUENCED BY SELECTION

A single experiment indicating the increase in resistance that may be obtained from selection alone will be reported here. In the summer of 1931 a small field planting of cuttings of highly resistant plants, well separated from any susceptible plants, set seed. The planting consisted largely of selections from susceptible common alfalfa, with a few plants of Ladak and of Turkistan origin. The open-pollinated seed from each individual plant represented was kept separate and plants from this seed were tested for resistance the following year, in some cases in comparison with a part of the original seed lots. The results of this test are summarized in table 5. In the 1930 test of the seed lots the plants were inoculated twice; in the 1932 retrial of those lots and in the test of the seed from the selections the plants were inoculated but once. The percentage of resistance given for the selections is an average of several trials in the same plot, the number of plants included ranging from 60 to 370. Differences between resistance in parent stock and selections in the first three instances and in Ladak do not, in the writer's opinion, represent a significant increase in resistance in the selection; but in all other cases the increase is regarded as highly significant. This result indicates that considerable increase in resistance can be obtained from some of the resistant plants selected by the procedure here described. Moreover, the resistant selections in this test appeared to have the general character of the stock from which they came and were not of the early-dormant Turkistan type. Additional selections from this plant material and from other sources are being made for a further test of the possibility of increasing resistance in strains from selected plants.

TABLE 5.—*Comparison of resistance in some highly susceptible seed lots with resistance in the $F_1$ population of selected resistant plants from those seed lots*

[Resistance is represented by the percentage of plants remaining uninfected at the end of a routine inoculation trial]

| Parent stock no. | Variety | Resistance of parent stock | | Resistance in $F_1$ population from numbered selected plants (1932) [b] [c] | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1930 [a] | 1932 [b] | Plant 1 | Plant 2 | Plant 3 | Plant 4 | Plant 5 |
| 1210 | Utah Common | 0.5 | | 11.7 | 8.7 | | | |
| 1225 | Kansas Common | .5 | 14 | 15 | | | | |
| 1245 | Utah Common | 1 | 22 | 22 | | | | |
| 1289 | Arizona Common | 1.4 | 3.4 | 23.5 | 16 | | | |
| 1300 | Montana Common | 1.4 | 3.4 | 25 | 16 | | | |
| 1301 | Kansas Common | 2.4 | | 40 | 23.3 | | | |
| 1302 | Do | 1.8 | 7 | 49 | | | | |
| 1303 | Do | 32.4 | | 35.3 | 38.7 | 37.5 | 66.6 | |
| 1304 | Do | 2.8 | 7 | 26.6 | 33.1 | 31 | 29.8 | 40 |
| 1334 | Ladak | 30 | | 36 | | | | |
| 1335 | Grimm | 1 | | 31 | | | | |
| 1356 | Turkistan | 24 | | 66 | 43.7 | 59.3 | | |

[a] Inoculated twice. [b] Inoculated once. [c] Average of several trials.

## SUMMARY

Alfalfa plants grown from seed from various sources have been inoculated at Madison, Wis., during 3 years for two purposes, namely, the comparison of resistance in the sources represented and the selection of resistant plants from which resistant strains may be developed.

Several procedures by which resistance in plants from different seed lots may be compared are described and evaluated.

Alfalfa from Turkistan and Ladak shows far more resistance than that from any other source from which seed has been tested thus far. However, occasional highly resistant plants are found from other sources.

On the basis of the routine inoculation used in the tests, resistant plants have been tentatively grouped in three classes, namely, immune, highly resistant, and resistant.

Resistance appears to be exhibited largely in the parenchymatous tissue of the plant through which the bacteria make little or no progress in resistant plants, and somewhat through the less rapid multiplication of the bacteria in the vessels.

No evidence has been found of morphological differences distinguishing resistant plants.

Increase in resistance in open-pollinated progeny from some of the selected plants has been obtained.


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# A GALL SIMILAR TO CROWN GALL, PRODUCED ON GYPSOPHILA BY A NEW BACTERIUM [1]

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## INTRODUCTION

Galls on the crown and roots of *Gypsophila paniculata* L. were first brought to the attention of pathologists of the Bureau of Plant Industry in the summer of 1932, when a grower submitted specimens of galls that had occurred in an ornamental-plant nursery in the eastern part of the United States. Galls on *Gypsophila* apparently were seen for the first time in 1931 by eastern growers of this important ornamental plant. However, neither the damage to plants nor the financial loss was extensive; consequently the growers did not at that time bring the disease to the attention of Federal workers interested in plant-disease problems.

In 1932, of 12 nurserymen who produced *Gypsophila paniculata* for the wholesale trade, only 2 were familiar with the disease, and one of these minimized his losses. He admitted that he understood the potential danger but stated that his loss had been less than 1 percent. The losses of the nurseryman who submitted the galled plants in 1932 amounted to about 25 percent.

A note regarding the discovery of the disease was published in the autumn of 1932.[2]

## THE DISEASE

The galls occur principally on grafted plants in the region of the graft. They are of a soft nodular type, ½ to 3 cm in diameter (fig. 1, *A*), and may extend around the greater part of the stem or root, eventually causing the death of the plant. It is the practice of the eastern *Gypsophila* growers to lift seedling plants in the fall to use later for grafting with the desired variety. Should their field plants be galled in the summer, they are worthless when dug.

Crown gall of ornamental plants, of vegetables, and of fruit trees, which is produced by *Bacterium tumefaciens* Smith and Town., is so well known and wide-spread that no surprise is manifested when a new host plant for the disease is found. Consequently, when the *Gypsophila* gall was received and examined and its outward appearance was observed to be very like crown gall, it was at first thought that *Gypsophila* was a new host for crown gall and that the disease was not new.

The routine work for identification, however, changed this idea. Cross sections examined under the microscope showed water-soaked areas and masses of bacteria streaming from the tissues, characters that are unlike crown gall. No nematodes or fungi were present. The disease was not crown gall, but there was a possibility that it might be related to one of the other bacterial plant tumors, namely,

[1] Received for publication Mar. 19, 1934; issued July 1934.

[2] BROWN, N. A. ANOTHER GALL-FORMING BACTERIUM. Phytopathology 22: 924-925, illus. 1932.

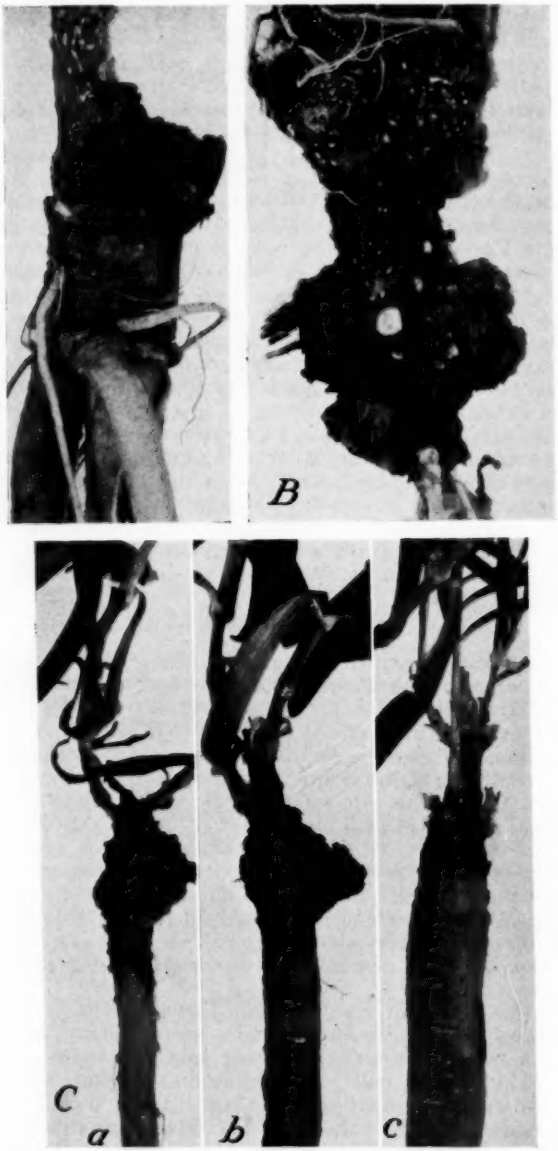


FIGURE 1.—*A, Gypsophila paniculata* gall from an Eastern State. *B, G. paniculata* gall 5 weeks old, produced by inoculating with an organism isolated from *A*. *C*, Seedling *G. paniculata* plants: *a* and *b*, Inoculated with 2 different colonies reisolated from gall *B*, which produced these galls in less than 1 month; *c*, control plant. Natural size.

the pocket disease of the sugar beet, produced by *Bacterium beticola* (Smith, Brown, and Townsend) Potebnia; the olive knot, produced by *Bact. savastanoi* E. F. Smith; the oleander gall, produced by *Bact. savastanoi* var. *nerii* C. O. Smith; or a canker of ash trees, produced by *Bact. savastanoi* var. *fraxini* Brown.

A further study of the structure of the *Gypsophila* gall, however, showed that it had features unlike these last-named tumors and could not be classed with them. There were no gum pockets in any of the *Gypsophila* galls received for examination. There were a few brown areas in some of them, but whether the discoloration was the beginning of the break-down of the gall tissue due to the invading organism or to a reaction of the gall tissue to the byproducts of this organism is not known. No browning occurred in the galls produced by inoculation. The *Gypsophila* galls are so soft that disintegration occurs very easily.

The galls were studied in cross section (fig. 2) and in the water-soaked areas motile bacteria were seen in the cells. Some of the cells were filled with them, others were partly filled, while many had none. A few of the tumor cells were packed with crystals. The cells near the periphery of the galls contained the greatest number of bacteria. The causal organism of crown gall has not been seen in the natural gall.

The structure of the *Gypsophila* galls was found to be much like that of crown gall. Nests of rapidly developing cells could be distinguished in which parenchyma and sclerenchyma cells were mixed irregularly (figs. 2, *A*; 3, *C*).

## ISOLATIONS AND INOCULATIONS

### COLONIES

An organism was isolated from several galls, the same type of colony appearing on the plates poured from each gall. The colonies were abundant and apparently consisted of the pathogene responsible for the disease. They appeared in 24 hours, were translucent white in reflected light, circular, 2 to 4 mm in diameter, slightly raised in the center, and finely granular (fig. 2, *B*). Buried colonies were mostly lens-shaped, but some were round. There were no irregular colonies. In 3 days they were a creamy yellow, 4 to 7 mm in diameter, and on thinly sown plates, 8 to 11 mm. Both rough and smooth colonies appeared on the plates isolated from the same gall, but the smooth colonies predominated. Both types of colonies were used for inoculations and produced galls of similar size, although the rough type produced them a little more slowly than did the smooth type.

By means of needle pricks grafted *Gypsophila* plants were inoculated at the crown and also on the stems with several of the isolated colonies. There was a beginning of gall formation at the crown in 7 to 9 days. In 2 weeks light-colored nodular galls 1 to 1½ cm in diameter showed at the crowns. Some of the inoculated plants were slow in showing infection, but in 3 weeks all had definite galls (fig. 1, *B*). When they were 2½ to 3 cm across, quite frequently disintegration began (fig. 4, *A*). Cankers, instead of galls, were produced on the inoculated aerial stems. On the 24 plants inoculated, the infection on crown and stem was 100 percent.

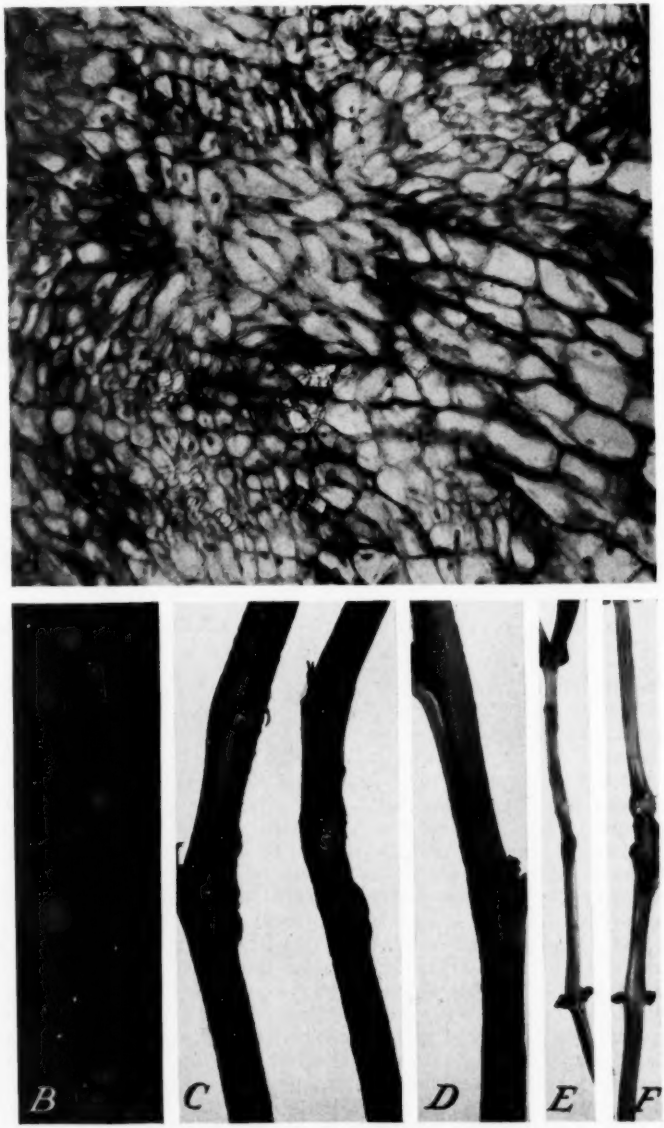


FIGURE 2.—*A*, Cross section of *Gypsophila paniculata* gall showing cell structure. *B*, Agar-plate colonies of the *Gypsophila* gall organism; natural size. *C*, Potato stems showing swellings; stems inoculated 3 weeks with *Gypsophila* gall organism that had previously passed through a potato stem and been reisolated; natural size. *D*, Control punctures on potato stem made at the same time as inoculations in *C*; natural size. *E*, *Saponaria vaccaria* inoculated with *Gypsophila* gall organism 4 days. *F*, *S. vaccaria* inoculated with *Gypsophila* gall organism 11 days. Organism is most active on this host, which is a relative of *Gypsophila*. *E* and *F* slightly reduced in size.

Roots of seedling *Gypsophila* plants inoculated at the crown likewise gave 100 percent infections. The galls formed about as rapidly as on the older grafted plants (fig. 4, *C*, *a*). Hot moist conditions favored gall development. Platings were made from the galls produced by inoculation, and the organism was recovered. *Gypsophila* plants inoculated with this reisolated strain developed galls of the same type as rapidly as did plants inoculated with the original strain (fig. 1, *C*, *a*, *b*, *c*).

At the time the *Gypsophila* plants were inoculated, stems of tomato (*Lycopersicon esculentum* Mill.) and potato (*Solanum tuberosum* L.), carnations (*Dianthus caryophyllus* L.), garden balsam (*Impatiens balsamina* L.), sugar beets (*Beta vulgaris* L.), Paris daisies (*Chrysanthemum frutescens* L.), nasturtiums (*Tropaeolum* L.), and other plants were inoculated with the same organism. No galls formed on any of them, but well-defined swellings occurred on the potato stems. Isolations were made from the potato swellings, the organism was recovered and outgrowths similar to the natural galls were obtained by inoculating *Gypsophila* plants with this potato isolation (fig. 3, *A*). Potato stems, inoculated with the potato isolation, developed swellings like those caused by the original organism, but neither galls nor cankers (fig. 2, *C*, *D*). Inoculations made into potato tubers attached to the plant at different stages of growth produced no outgrowths.

The common hosts of *Bacterium tumefaciens*, such as Paris daisy, sugar beet, *Ricinus* L., geranium (*Geranium* L.), and garden balsam, did not prove susceptible to the *Gypsophila* gall organism when they were inoculated with it, nor did *Bact. tumefaciens* produce any trace of outgrowth on roots or stems of *Gypsophila paniculata* (fig. 4, *C*, *b*, *c*). The roots and stems of *G. paniculata* were also inoculated with the olive-knot organism (*Bact. savastanoi*) and the ash-canker organism (*Bact. savastanoi* var. *fraxini*) with negative results.

### FILTRATES AND PLEOMORPHIC FORMS

The juice of crushed *Gypsophila* galls was passed through Chamberland L 3 filters, and *Gypsophila paniculata* and *Lychnis chalcedonica* L. plants were inoculated with the filtrate. No galls resulted. Filtrates from beef-bouillon cultures were also used for inoculations, with the same result. A portion of the filtrates was held in sterile tubes for several weeks, then cultured repeatedly on hardened agar plates, according to the technic of Hauduroy [3] and of Hadley.[4] With this procedure the filtrates passed through the granular and coccus stages and later reached the normal rod form again, but when the cultures arrived at the rod form the ability to infect the *Gypsophila* plants was lacking. The cultures would not produce galls.

The writer had tried out the method previously with three different strains of *Bacterium tumefaciens*, namely, the hop, daisy, and peach strains. Neither the crushed-gall filtrates nor the beef-bouillon-culture filtrates of the three strains produced galls when inoculated into susceptible plants. Portions of the sterile filtrates were held in tubes for a few weeks to a few months, then cultured for some time on hardened agar plates. From the granules, the coccus form developed, and later from the coccus the normal rods. Inoculations were made into young

[3] HAUDUROY, P. LES ULTRAVIRUS ET LES FORMES FILTRANTES DES MICROBES. 392 pp. Paris. 1929.

[4] HADLEY, P., DELVES, E., and KLIMEK, J. THE FILTRABLE FORMS OF BACTERIA. I. A FILTRABLE STAGE IN THE LIFE HISTORY OF THE SHIGA DYSENTERY BACILLUS. Jour. Infect. Diseases 48: 1–159. 1931.

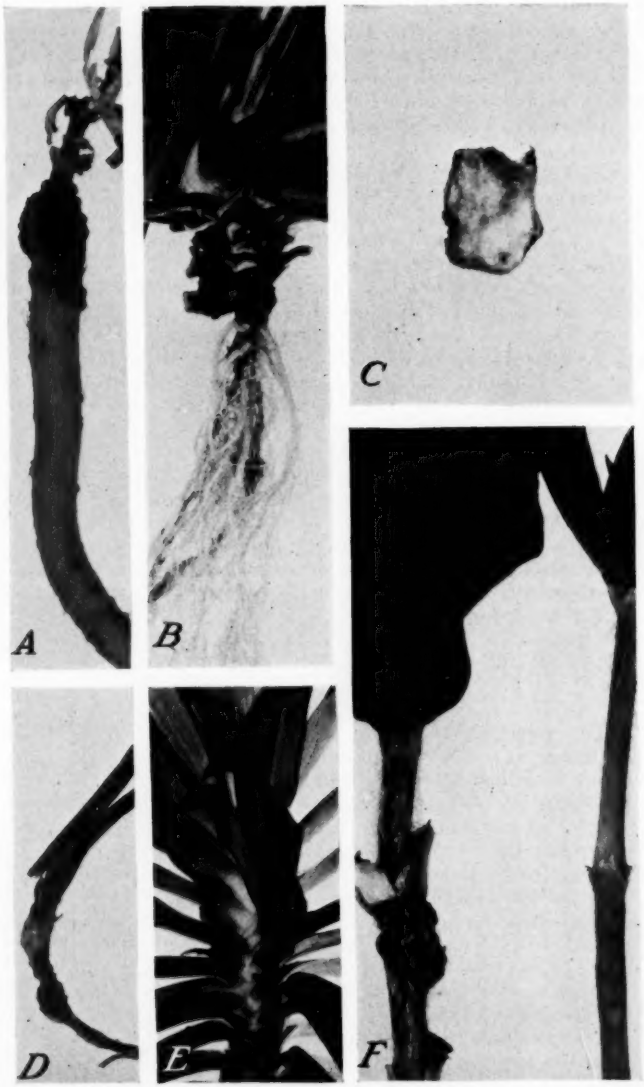


FIGURE 3.—*A*, Gall on seedling *Gypsophila paniculata*, produced by inoculation with a strain of the organism reisolated from a potato stem on which a swelling was produced but not a gall. The potato reisolation, however, produced galls on *Gypsophila* root. Photographed 5 weeks after inoculation. *B*, Gall on *Silene armeria* produced by inoculation with the *Gypsophila* gall organism; time, 2 months. *C*, *Gypsophila* gall 1 month old cut across to show internal structure. *D*, Gall on *Dianthus plumarius* (garden pink) produced by inoculation with the *Gypsophila* gall organism; time, 2 months. *E*, *Dianthus barbatus* (sweet-william) inoculated with *Gypsophila* gall organism 2 months; no infection. *F*, At left gall on *Lychnis chalcedonica* produced in less than 1 month by inoculation with the *Gypsophila* gall organism; at right, control punctures on *L. chalcedonica*. All natural size.

susceptible plants with both coccus and rod forms, but no trace of crown gall resulted.

With the *Gypsophila* gall filtrates the transition stage from coccus to rod came more quickly than with the filtrates of *Bacterium tumefaciens*, and it was hoped pathogenicity accompanied this less tedious manipulation. It did not prove to be the case, however, as no infection followed inoculations into susceptible plants.

## THE CAUSAL ORGANISM

### CULTURAL CHARACTERS

BEEF-INFUSION AGAR PLATES.—White colonies are visible in 24 hours after pouring plates from macerated gall tissue incubated at 22° to 25° C. In 48 hours they are deep cream to wax yellow and range from 2 to 4 mm in diameter; they are smooth, circular with entire margin, shining, convex, a little thicker in the center. In 4 days the colonies on thinly sown plates are 4 to 11 mm across, and in some there is a margin. At 6 days the color is mustard yellow; later, primuline yellow.[5]

After the organism has been cultured a few weeks in artificial media, plates poured from a 1-day beef-bouillon culture may show more rough than smooth colonies. These are much the same as the colonies that appear from the isolation material. Occasionally there is a rough colony that has a high convoluted surface. The color of week-old cultures is primuline yellow.

BEEF-INFUSION AGAR SLANTS.—There is a thin spreading growth, usually papillate but sometimes smooth, on beef agar slants in 24 hours. Under the hand lens it has a metallic luster on beef agar. The pH is 6.8 at temperatures of 25° to 30° C. At 4 days growth is abundant, butyrous, translucent; at 7 days the metallic luster has disappeared and there are many crystals. The color of the growth is Naples yellow.

BEEF-INFUSION BOUILLON.—Clouding is prompt, being quite definite in 7 hours at 34° C.; at 30° there is good clouding in 24 hours; in 48 hours a yellow pellicle has formed which falls readily.

THAXTER'S POTATO-DEXTROSE AGAR SLANTS.—The growth is spreading but not so rapid as on beef agar; it is rough, butyrous, cream-colored, and continues so when a week old.

POTATO CYLINDERS.—There is a thin cream-yellow growth in 1 day; it is still scanty at 6 days, with the color buff-yellow, and the potato is slightly discolored. After 30 days the color is Naples yellow, but the medium has not darkened further.

COHN'S SOLUTION.—Growth is rapid and heavy in Cohn's solution, and a complete pellicle forms with larger irregular crystals hanging from it. At first the pellicle is white but changes in 7 days to Naples yellow. The medium becomes cream-colored and has a yellow precipitate.

USCHINSKY'S SOLUTION.—Growth is prompt in this medium, and there is a heavy white pellicle in 2 days; in 12 days the pellicle is cream-colored and the medium Naples yellow.

FERMI'S SOLUTION.—Growth occurs readily but is not so heavy as in Uschinsky's solution. There is a white pellicle in 2 days, which changes to mustard yellow in 12 days.

### PHYSIOLOGIC CHARACTERS

LIQUEFACTION OF GELATIN.—No liquefaction begins in beef-gelatin stabs until the cultures are a month old. The liquefaction continues slowly and is not completed until after 4 months. The cultures were kept at 14° to 15° C. and three different lots of beef gelatin, having pH 6.5, 7.0, and 7.3, respectively, were used.

Colonies on beef-gelatin plates are cream-colored until 6 days old, when they become mustard yellow. Both smooth and rough colonies occur on all plates, with the rough type more abundant; they are translucent in reflected light, transparent in transmitted light. In 3 weeks there are little hollows around the colonies, and in 4 weeks liquefaction is definitely visible but continues very slowly. In heavily seeded plates the gelatin is entirely liquefied in 4 months; in sparsely seeded plates the liquefaction extends 1 cm beyond the colony. Feathery branched crystals are formed on the plates and in the stab cultures.

[5] The color readings in this paper are based on the colors in the following publication: RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D.C. 1912.

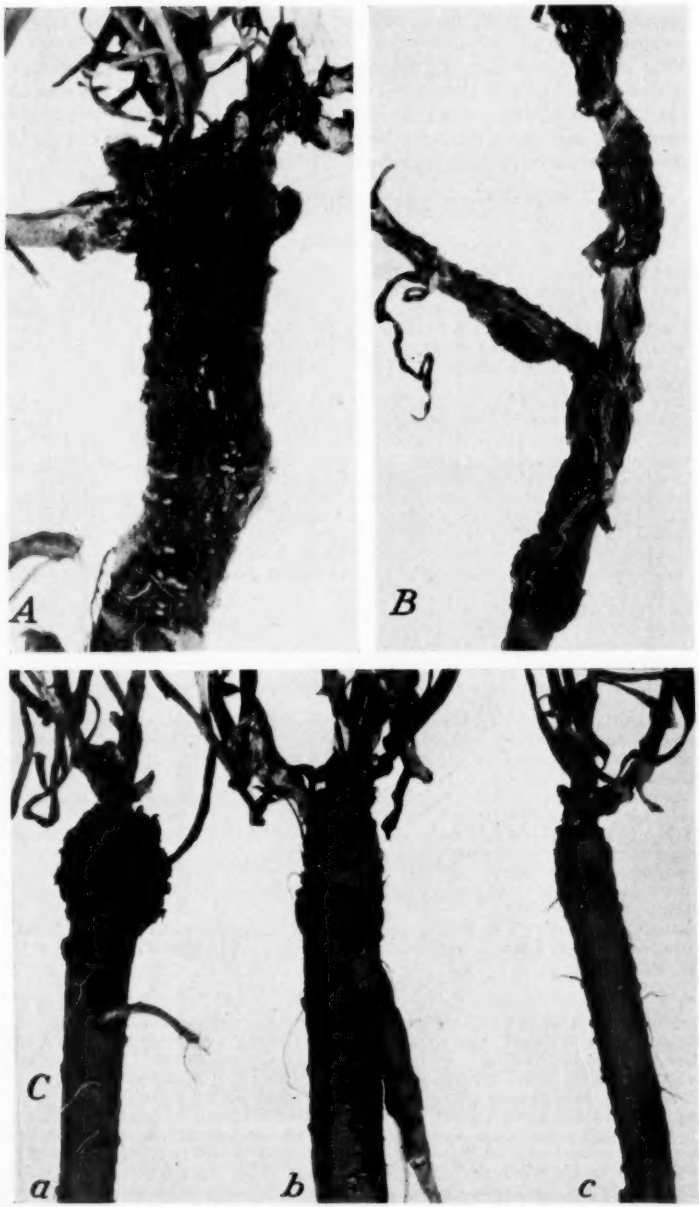


FIGURE 4.—*A*, Gall on *Gypsophila paniculata*, mostly rotted off. Crown was inoculated with *Gypsophila* gall organism July 23, 1932. Photographed August 30, 1932. *B*, Cankers, not galls, produced on *G. paniculata* stems, by inoculation with tne *Gypsophila* gall organism; time, 3 months. *C*, Seedling *G. paniculata* roots inoculated with various organisms 2 months: *a*, with *Gypsophila* gall organism; *b*, with *Bacterium tumefaciens* hop strain, no infection; *c*, with *Bact. tumefaciens* daisy strain, no infection. All natural size.

MILK.—Coagulation of milk occurs at 9 days, with very little whey. A light straw-colored pellicle is visible at 6 days. The curd digests slowly, beginning at 17 days and being completed in 90 days, at which time the color of the milk is tawny.

BLOOD SERUM.—A very good growth takes place on blood serum, but there is no liquefaction. The mustard-yellow color at 2 days becomes the deeper primuline yellow at 12 days. The blood serum grays a little at the base. Cultures 2 months old show no trace of liquefaction.

REDUCTION OF LITMUS.—There is a dull pink color throughout litmus-milk cultures in 24 hours, and the original color, pale aniline lilac, is changed to pale lobelia violet; in 48 hours this color is still duller except at the surface of the liquid. In general the color change in comparison with the uninoculated tubes is slight. In 8 days the litmus has faded to lavender-gray and in 10 days it is reduced, the color now being tilleul-buff; there is a yellow pellicle and yellow precipitate of the growing organism. Coagulation usually takes place on the ninth day, sometimes on the eighth day, after litmus milk has been inoculated. Digestion of the curd is slow, not being completed before 3 months. The medium at this time is dull reddish purple, and the organism is still alive.

REDUCTION OF NITRATES.—Nitrates are reduced to nitrites. Tests were made on nitrate-bouillon cultures 3 and 5 days old, with the sulphanilic acid α-naphthylamine test. There was a good red color in all the tubes, indicating the presence of nitrites. Other cultures when 25 and 30 days old were tested with the same result.

INDOL PRODUCTION.—No indol is produced. Tests were made on the organism growing in tryptophane broth at 3, 5, and 30 days, respectively, with the Ehrlich-Böhme method. *Bacillus coli* (Escherich) Migula, which produces indol, grown as a control and tested at the same time, gave a good pink color, a positive test. The *Gypsophila* gall organism showed no pink color.

FERMENTATION OF CARBOHYDRATES.—The organism is not a gas former. It was tested in fermentation tubes in the presence of the following carbon compounds: Dextrose, saccharose, lactose, glycerin, and mannite. A 1-percent solution of each was made in a 1-percent water solution of Difco peptone. Besides heavy growth in the open arm of each tube there was growth in the closed arm of the tube with each compound except glycerin. No gas was produced. Acid was produced in all the solutions but that of lactose. A second test was made with the same result. The pH readings were taken just before inoculation with the *Gypsophila* gall organism, also 20 and 27 days after, as shown in table 1.

The same carbohydrates added to synthetic agar with brom cresol purple as indicator, made according to the formula given in the Manual of Methods,[6] were also tested for fermentation. Growth occurred promptly, as did the acid reaction with saccharose, dextrose, glycerin, and mannite, the yellow color change in the purple medium beginning in 18 hours and being complete in 48 hours. There was growth in the lactose cultures but no color change.

TABLE 1.—*Acid production by the Gypsophila gall organism after 20 and 27 days of growth in 1-percent sugar solutions added to 1-percent Difco peptone*

[Acidity indicated by pH readings]

| Number of days of growth | pH of solution containing peptone and indicated carbohydrate | | | | | pH of plain peptone water |
|---|---|---|---|---|---|---|
| | Dextrose | Lactose | Saccharose | Mannite | Glycerin | |
| 0 | 6.5 | 6.5 | 6.7 | 6.7 | 6.5 | 6.6 |
| 20 | 4.2 | 7.0 | 5.0 | 5.0 | 6.4 | 7.4 |
| 27 | 4.4 | 6.8 | 5.0 | 4.9 | 6.5 | 7.3 |

DIASTATIC ACTION.—There is no reduction of starch. On starch-agar plates streaked with the organism and tested at 5, 9, 12, and 16 days, respectively, there was no clear zone in the medium.

AMMONIA PRODUCTION.—The organism produces ammonia. Tests were made with old and young beef agar, beef bouillon, and peptone-water cultures, using

[6] SOCIETY OF AMERICAN BACTERIOLOGISTS, COMMITTEE ON BACTERIOLOGICAL TECHNIQUE. MANUAL OF METHODS FOR PURE CULTURE STUDY OF BACTERIA. 130 pp., illus. Geneva, N.Y. 1930.

filter paper saturated in Nessler's solution and suspended in the tubes. When the cultures were heated in a water-bath, browning of the paper began immediately. In cultures 2 weeks old the brown color was much more pronounced than in the 3- and 5-day cultures.

HYDROGEN SULPHIDE PRODUCTION.—The organism may produce a trace of hydrogen sulphide. Agar, beef-bouillon, and potato-cylinder cultures were tested by suspending lead acetate paper in the culture tubes. When the beef-bouillon cultures were 8 days old there was slight darkening at the tip ends of the paper, indicating hydrogen sulphide production; there was slightly more darkening of the paper when cultures were 2 weeks old. There was no darkening of the paper in the other cultures.

The organism was then grown on lead acetate agar. A heavy yellow growth occurred on the red agar, but there was no dark line or any browning indicating the presence of hydrogen sulphide. A second and third test with the lead acetate agar was made which likewise showed no hydrogen sulphide production.

TOLERATION OF SODIUM CHLORIDE.—The organism grows in pH 6.5 beef-infusion bouillon containing 6, 7, 8, or 9 percent of sodium chloride. There is no growth in beef bouillon containing 10 percent of sodium chloride.

OXYGEN RELATIONS.—The organism is a facultative anaerobe. In tests made with shake cultures of agar and gelatin, tiny clumplike colonies grew throughout the medium. When long sterile cover glasses were dropped on hardened agar plates streaked with the organism, growth was more abundant at the edges of the cover glass, but it extended inside the edges, showing the anaerobic tendencies. In agar and gelatin stab cultures it grew at once at the bottom of the tube, and in synthetic-dextrose-indicator-agar shake cultures, the purple color of the medium was changed to red at the bottom of the tube as quickly as at the surface of the medium.

THERMAL RELATIONS.—The organism grows at temperatures ranging from 5° to 40° C. The optimum temperature is about 34°; it does not grow at 0° nor at 42° and only faintly at 5° and 40°.

The thermal death point is between 52° and 53° C., when fresh beef-bouillon cultures, pH 7.0, are exposed in a water bath for 10 minutes.

GROWTH IN BEEF BOUILLON.—The best growth in peptone-beef-infusion bouillon takes place at pH 6.5 to 6.7, although the organism has a wide range, extending from pH 5.1 to 9. There is no growth at pH 4.9 or 9.1. At pH 5.1 there is only a faint growth; at pH 9 there is a fair amount of clouding with pellicle.

EFFECT OF DESICCATION.—The organism is only slightly resistant to drying. Sterile cover glasses smeared with young beef-bouillon cultures and dried at room temperatures (24° to 27° C.) were dead in 5 days.

EFFECT OF FREEZING.—The organism can withstand freezing temperatures for more than 45 days. Immediately after being transferred, beef-agar and beef-bouillon cultures were placed at temperatures of −21.7° to −23.9° C. Some were removed after 7, 9, 12, and 45 days. All showed typical growth within 1 day after the medium melted.

LONGEVITY.—The organism lives for 7 to 8 months in sterile milk and beef bouillon, pH 6.8, at room temperature of 22° to 30° C., whereas it dies on beef agar slants, in Cohn's, Fermi's, and Uschinsky's solutions, after 4 months at the same temperatures. Sterile-milk and beef-bouillon cultures kept at 14° are alive after 11 months.

VIRULENCE.—The organism remains virulent for more than a year. Fifteen months after isolating, transfers descended from the original isolation, including a smooth and rough colony, were inoculated into *Gypsophila paniculata* plants. In 7 days the galls were forming and continued to grow rapidly.

CHROMOGENESIS.—On beef agar the color of this organism is at first a light cream that changes to a Naples yellow in a few days. It is much the same in other solid media. Later the color may be mustard yellow or primuline yellow.

## MORPHOLOGY

Grown on beef-infusion agar, the *Gypsophila* gall organism is a short rod with rounded ends growing singly, in pairs, and occasionally in chains of four to several elements; in rare cases there have been more than 25. Grown on beef agar for 1 day and stained with carbol fuchsin, the size is 0.5$\mu$ to 1.2$\mu$ long by 0.3$\mu$ to 0.8$\mu$ wide. Grown on the same medium for 2 days and stained with gentian violet, the

size is 0.4$\mu$ to 1.03$\mu$ long by 0.2$\mu$ to 0.62$\mu$ wide. The organism is motile on beef agar and in beef bouillon and its motility was demonstrated by staining with Casares-Gil flagella stain. There are several flagella, all bipolar. Capsules are formed, as was shown by staining young beef agar cultures with Ribbert's capsule stain. The tests for endospores showed none.

### STAINING RELATIONS

The organism stains well with gentian violet and carbol fuchsin. It is not acid-fast and is Gram-negative. (Hucker's modification of Gram was used.)

### TECHNICAL DESCRIPTION

**Bacterium gypsophilae, sp. nov.**

A motile rod 0.4$\mu$ to 1.2$\mu$ long and 0.2$\mu$ to 0.8$\mu$ wide, with several bipolar flagella; capsules present, no spores; Gram-negative, not acid-fast; colonies on beef-infusion agar are circular, either smooth or rough, yellow, butyrous; clouds beef-infusion bouillon heavily in 18 hours; liquefies gelatin slowly, but not blood serum; is facultative anaerobic; coagulates milk; reduces litmus in 9 to 12 days; grows well in Uschinsky's and Fermi's solutions and unusually well in Cohn's solution; reduces nitrates; produces ammonia and a trace of hydrogen sulphide but no indol; no diastatic action; survives cover-glass drying only 4 days; acid without gas produced with saccharose, dextrose, maltose, mannite, but not lactose, and only a slight amount with glycerin; the optimum temperature for growth is over 30° C., the maximum is 40°, the minimum is 5°; thermal death point is between 52° and 53°; optimum reaction for growth is from pH 6.5 to 6.7, limits of growth from pH 5.1 to 9.0; in beef bouillon and in sterile milk lives 8 months at 22° to 28°, over 11 months at 14°; stains readily with carbol fuchsin and gentian violet; pathogenic to *Gypsophila paniculata* and some of its relatives, producing galls on the crown and root, and cankers on the stem.

### COMPARISON WITH BACTERIUM BETICOLA

Because of certain points of resemblance between *Bacterium gypsophilae* and *Bact. beticola* and the lesions caused by them, a study of certain cultural, physiologic, and morphologic characters of these organisms was made. A comparison of these characters is shown in table 2.

## NATURAL INFECTION AND CONTROL

The *Gypsophila* gall organism is selective in its host plants, as only related plants have been found susceptible to it; however, there are also related plants which are not susceptible. The limitation of this gall-forming ability differs from that of the crown-gall organism, which produces galls on many unrelated plants. The hosts susceptible to the *Gypsophila* gall organism are *Lychnis chalcedonica* (fig. 3, *F*), *Dianthus plumarius* L. (fig. 3, *D*), *Silene armeria* L. (fig. 3, *B*), and *Saponaria vaccaria* L., which is the weed soapwort (fig. 2, *E* and *F*). This last relative seems to be more susceptible to the organism than *Gypsophila paniculata* itself, for infection begins in 3 to 4 days after inoculation and galls are formed very rapidly. It may be that this weed is the natural host of the *Gypsophila* gall organism and it would be advisable not to allow it to grow in the neighborhood of *Gypsophila* plants. Another relative, *Saponaria ocymoides splendens* Hort., is slow to become infected, as the galls did not begin to form on young plants until nearly 3 weeks after inoculation. The relatives, *Cerastium tomentosum* L., *Tunica saxifraga* Scop., *Spergula pilifera* DC., *Dianthus barbatus* L., which is the common sweet-william

(fig. 3, *E*), and the greenhouse carnations are not susceptible. It is an interesting fact that the carnation is not infected by the *Gypsophila* gall organism, for *Bacterium tumefaciens* produces galls thereon quite readily, and occasionally natural *Bact. tumefaciens* galls are found on it. To be quite certain that carnation plants could not be infected by the *Gypsophila* gall organism, inoculations were made at different times of the year and in different growing stages of the plant. Other hosts that did not prove susceptible are sugar beet, tobacco (*Nicotiana tabacum* L.), *Ricinus communis* L., Paris daisy, *Impatiens balsamina*, tomato, geranium, rose (*Rosa* L.), *Bryophyllum pinnatum* Kurz, nasturtium, and two monocotyledons, amaryllis (*Amaryllis* L.) and calla (*Zantedeschia aethiopica* (L.) Spreng.). As stated previously decided swellings but no galls formed on the potato stem.

TABLE 2.—*Comparative cultural, physiologic, and morphologic characters of Bacterium gypsophilae and Bact. beticola*

| Character compared | *Bacterium gypsophilae* | *Bacterium beticola* |
|---|---|---|
| Colonies in beef-agar plates | Circular; none irregular; butyrous; white first day; deep cream to yellow, 4 to 8 mm in diameter, in 4 days. | Circular; some irregular; viscid; buff-colored first day; yellow, 4 to 6 mm in diameter, in 4 days. |
| Cohn's solution | Rapid, heavy growth | No growth. |
| Liquefaction of gelatin stabs | Begins after 30 days; complete after 4 months. | Begins after 7 to 8 days; complete in 20 to 30 days. |
| Hydrogen sulphide production | A trace to none | Rapid and good production. |
| Indol production | None | None. |
| Reduction of nitrates | Nitrates reduced | Nitrates reduced. |
| Reduction of litmus milk | Complete in 9 to 10 days; milk coagulated in 8 to 9 days. | Complete in 20 to 30 days; milk coagulated in 10 to 20 days. |
| Gas production | None | None. |
| Acid produced with dextrose, saccharose, glycerin, and mannite | Acidity produced | Acidity produced. |
| Acid produced with lactose | None | None. |
| Ammonia production | Ammonia produced | Ammonia produced. |
| Diastatic action | None | Starch reduced. |
| Relation to oxygen | Facultative anaerobic | Aerobic. |
| Relation to acid and alkali | pH range 5.1 to 9.0 | pH range 4.8 to 9.1. |
| Temperature relations | Grows from 5° to 40° C; optimum, about 34°; thermal death point, 52° to 53°. | Grows from 1.5° to 39° C.; optimum, about 29°; thermal death point, 51° to 52°. |
| Survives cover-glass drying | 4 days | 7 days. |
| Color | Ranges from white to yellow | Ranges from buff to yellow. |
| Size | 0.4$\mu$ to 1.2$\mu$ long; 0.2$\mu$ to 0.8$\mu$ wide | 0.6$\mu$ to 2$\mu$ long; 0.4$\mu$ to 0.8$\mu$ wide. |
| Gram negative or positive | Negative | Variable. |
| Pathogenicity | Produces galls on *Gypsophila paniculata* but not on sugar beets. | Produces galls on sugar beets but not on *Gypsophila paniculata*. |

The *Gypsophila* gall organism produces cankers on stems of *Gypsophila paniculata*, fair-sized cankers forming in less than 1 month after inoculation, large ones in 3 months (fig. 4, *B*). On *Lychnis chalcedonica* stems the infection is of the typical gall type. *Lychnis* crowns inoculated in November in the greenhouse developed galls, which rotted away during the winter. In the spring, when growth started, new galls formed which became larger than the original ones. This occurred with inoculated *G. paniculata* plants also, although the galls were not so soft and did not disintegrate so easily. The organism, like other organisms that produce galls, is a wound parasite, this one getting into the plant from the soil through imperfect grafting or through cultivation wounds. The disease occurs on lands that have been manured and on those that have not.

The rapid development of galls at the crown produces the death of some of the stems, and if the girdling is complete the death of

the entire plant follows. A small gall which has not caused any apparent trouble to the plant may be a decided menace to others later. The method used for propagating *Gypsophila* plants is to graft a desirable variety on seedling roots. If this variety is galled the organism is carried over to the young seedling roots and galls develop. Because of the sensitiveness of the gall organism to weak solutions of mercuric chloride, control measures can be carried on at the time of grafting to reduce the amount of disease occurring in the field. The roots should be dipped in a 1:1,000 mercuric chloride solution for 1½ to 2 minutes to kill the *Gypsophila* gall bacteria that may be on the surface; then with a disinfected knife a well-matched graft should be made and bound with nursery tape.

The sensitiveness of the organism to weak solutions of mercuric chloride was tested out by the poured-plate method. A fresh beef-bouillon transfer was exposed to 1 cc of a 1:1,000 solution of mercuric chloride for various lengths of time and then plates were poured, carrying over a loop of the exposed culture to each agar plate. No *Gypsophila* gall colonies appeared on the plates exposed over 1½ minutes to the mercuric chloride solution. As *Gypsophila paniculata* plants are not very sensitive to a 1:1,000 solution of mercuric chloride they can be treated with it to kill the gall organisms that may be present on the surface. Twenty-five seedling plants 3 to 6 inches tall were soaked in the solution for 1½ minutes, 25 for 3 minutes, 25 for 5 minutes, and 25 for 10 minutes. There was no appreciable injury to the plants from this treatment, even in the 10-minute lot.

In planting out seedlings and grafted plants, in weeding them and in loosening the soil about them during the summer, care should be taken to avoid wounding, for the organism may be present in the soil and may enter the plant through some tiny wound.

## SUMMARY

An outbreak of an infectious gall disease occurred in 1932 in an ornamental-plant nursery in the eastern part of the United States. The galls were on the crown and roots of *Gypsophila paniculata*. They were soft and nodular; some were flat and spreading, others globular.

The *Gypsophila* galls when developing do not have the fissures or pockets that occur in developing galls of pocket disease of sugar beets and in those of olive knot. Growth of the galls is favored by hot moist weather, which also favors the disintegration of the old galls, releasing more organisms into the soil. Imperfect grafting and cultivation wounds allow entrance of the pathogene into susceptible tissue. Galls weaken the plants, producing defoliation and death of stems and, where girdling is severe, death of the plants.

An organism, which could be seen readily in sound gall tissue under the microscope, was isolated from the outgrowths and produced galls when inoculated into healthy plants of *Gypsophila paniculata*.

Galls were also produced by inoculation on several species related to *Gypsophila*, but the organism did not produce galls on the sugar beet, which is the host of the pocket disease, nor on such common hosts of the crown-gall organism as the Paris daisy, *Ricinus*, or geranium.

For the pathogene, which is a yellow, polar-flagellate organism apparently unlike any other known gall-producing organism, the name *Bacterium gypsophilae* is proposed. A description of its cultural, physiologic, and morphologic characters is given.

A comparison has been made between the new gall organism and *Bacterium beticola*, the organism causing the pocket disease of sugar beets.

A study has been made of conditions governing the natural occurrence of infection, and methods of controlling the disease are suggested.

# EXPERIMENTS ON IAROVIZING CORN [1]

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## INTRODUCTION

The methods of iarovization advocated by the Russian workers fall into two distinct groups: The low- and the high-temperature treatments. The low-temperature treatments have been known for a long time and are well established experimentally, as is evidenced by the work of Klippart (*3, p. 757*),[3] Lysenko (*4*), McKinney and Sando (*6*), and others. The high-temperature treatments are less well established. They have been advocated as a very effective agent in the hastening of sexual maturity in the short-day plants. If the results reported by the Russians are universally confirmed, the process of iarovization might be expected to play a very important role in certain temperate regions. Iarovization of corn in the United States, however, even if effective, does not appear to offer any great commercial possibilities. On the other hand, it might have considerable value in certain physiologic and genetic experiments.

## THE PROCESS OF IAROVIZATION

Originally the term "iarovization" (vernalization) was applied only to the low-temperature treatment of winter wheat to induce jointing and hasten sexual maturity. At the present time the term is generally applied to any treatment having as its object the hastening of sexual maturity.

The requirements for different crops differ in the duration of the treatment, the temperature, and the moisture content of the seeds. A summary table is presented by McKinney and Sando (*7*) listing the requirements for a few crops. All of the high-temperature treatments, according to the Russian workers, must take place in darkness.

## MATERIALS AND METHODS

The experiments reported in this paper were conducted at the Arlington Experiment Farm, Rosslyn, Va., in 1933. Eight hybrids, 9 inbreds, and 1 variety of corn and 1 strain of teosinte were included. The variety of corn used was Gaspé, one of the earliest known. The hybrids and 7 of the inbreds are adapted to Corn Belt conditions. Of the remaining inbreds one is a derivative of the Garrick variety which is adapted to the South, and the other is a type called "Cuzcoid" because of its resemblance to the varieties from Cuzco, Peru. This last is a simple Mendelian recessive and in the segregating prog-

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[2] The writer acknowledges his indebtedness to H. H. McKinney and W. J. Sando for the use of temperature-control facilities and greenhouse space and for helpful suggestions during the progress of these investigations.

[3] Reference is made by number (italic) to Literature Cited, p. 1120.

enies so far tested has required about twice as long to reach the reproductive stage as its normal sibs. Sexual maturity in this strain has not been hastened by exposure to a 10-hour day.

A further indication of the range of season represented by the inbreds in this test may be obtained from the following comparison. The earliest strain, 616, shed pollen 60 days after planting. Cuzcoid, the latest strain, was killed by frost October 26, 160 days after planting, when less than one-tenth of the plants had tasseled. At this time it had an average of 38 nodes per plant.

All of the seed used was soaked in a 0.5-percent solution of Uspulun for 2 hours and then rinsed. They were then soaked for 9 hours in a weak salt solution of about the same molecular concentration as tap water and were then dried to approximately the moisture content (30 percent) recommended. This method was found to result in much more uniform germination than adding stated quantities of water as recommended by Lysenko (*4*). One complete set of the samples was then placed in light-proof bottles in a constant-temperature chamber and held at 75° F. for 14 days. Duplicates of some of the samples were subjected to the same temperature conditions, but exposed to the normal day or to continuous light (normal day plus artificial illumination). One lot was held at a temperature of 38° F. for the same period. At the end of the 14-day period all of the samples were planted in the field. In those samples in which germination had progressed the farthest the radicles were approximately 5 to 8 mm and the plumules 5 mm long. Molds, particularly *Penicillium*, occurred in some seed lots, but no visibly infected seeds were planted.

The iarovized material was hill-checked in some cases in comparison with sprouted seed, and in other cases with dry seed of the same sort. In some instances, the difference due to the slight hastening of emergence because of the sprouted condition of the iarovized seed persisted and was reflected in a slightly earlier tasseling and silking (table 2, items 3 and 6). This should not be a serious source of error in experiments of this kind, as a slight advantage from this cause would be insignificant as compared with the marked acceleration which must result from iarovization if the method is to be commercially practical.

Dates of germination, pollen shedding, and silking were recorded for the plants in all perfect hills. In addition, percentage of germination, number of nodes, plant heights, and plant yields were obtained in most cases. All of the data reported are based on comparisons between plants of the same strain growing in the same hill.

## EXPERIMENTAL RESULTS

The effect of the treatments on field germination and on emergence of the inbreds is shown in table 1. In every case iarovization resulted in a marked reduction in germination and in 13 of the 14 cases delayed emergence. The germination of the iarovized seed was so poor and seedling mortality following germination so great that no adequate data on maturity are available. For the three strains A, 324, and 540, which had the highest germination percentage and for which meager data are available, there was no indication of a hastened maturity following the treatments.

TABLE 1.—*Effects of iarovization on field germination and emergence in 14 inbred strains of corn*

| Inbred strain no. | Condition of check | Germination | | Mean difference between iarovized and check seed lots in time to emergence [a] |
|---|---|---|---|---|
| | | Iarovized | Check | |
| | | *Percent* | *Percent* | *Days* |
| 616 | Sprouted | 35.0 | 97.5 | 1.4 |
| A | do | 70.0 | 97.5 | 1.3 |
| 324 | do | 75.0 | 100.0 | .9 |
| 420 | do | 55.0 | 95.0 | 2.0 |
| 420 [b] | do | 32.5 | 97.5 | 1.7 |
| 420 [c] | do | 7.5 | 97.5 | 2.5 |
| 461 | do | 22.5 | 97.5 | 1.8 |
| 461 [b] | do | 25.0 | 95.0 | 1.7 |
| 461 [c] | do | 40.0 | 100.0 | 2.0 |
| 461 | Dry | 20.0 | 97.5 | 1.7 |
| 540 | Sprouted | 80.0 | 97.5 | .3 |
| 119–11–b | do | 22.5 | 92.5 | 2.0 |
| 207–37 | do | 35.0 | 72.5 | −.4 |
| Cuzcoid | do | 54.0 | 83.0 | +.5 |

[a] Positive differences indicate the iarovized lot emerged later than the check. In the column headed emergence a positive difference indicates that the iarovized lot required more days to emerge than the control.
[b] Iarovized in light (day only).
[c] Iarovized in light (continuous).

With the more vigorous material, which includes the hybrids and the variety Gaspé, there was a slight but consistent reduction in germination as a result of iarovization, as shown in table 2. The time required for germination shows no consistent differences resulting from the treatments. Only three of the mean differences are significant. In two of these, iarovization appears to have had an accelerating effect, but in both the check seed was dry and ungerminated. In one instance there is a significant retardation. In this paper, differences have been considered statistically significant when $P$ is 0.02 or less. Such differences are italicized in table 2.

In 8 of the 15 comparisons, plants from iarovized seed shed pollen significantly before the checks, but in only 5 cases was there a significant difference in silking. This is in accord with general observations that pollen shedding is influenced to a greater extent by environment than is silking. There is a fairly high positive correlation between the days required for emergence and for pollen shedding. Substantially the same degree of correlation exists in both the iarovized and control lots. It should perhaps be emphasized that while in several cases hastening of sexual maturity is statistically significant, in no case is the acceleration of any significance agronomically.

The numbers of nodes visible at maturity were fewer in the plants from iarovized seed in all of the 11 comparisons involving iarovized *v.* noniarovized seed and of which counts were made. Only 7 of the 11 differences are statistically significant, but 11 deviations of like sign would be expected only once in 2,048 trials as a result of sampling error alone. Counting nodes visible at maturity is not satisfactory where the absolute number is wanted. In the present case, however, the interest lies in the relative difference between paired plants of successive hills, and the visible number of nodes is just as satisfactory a basis for comparison as the absolute number.

TABLE 2.—*The effects of iarovization on various agronomic characters in 8 hybrids and 1 variety of corn*

[Differences for which $P$ is less than 0.02 are italicized]

| Hybrid or variety | Condition of check | Field germination | | Mean differences between iarovized and control plantings in— [a] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Time to— | | | Nodes | Plant height | Ears | Weight of shelled grain per plant |
| | | Iarovized | Check | Emergence | Pollen shedding | Silking | | | | |
| | | *Pct.* | *Pct.* | *Days* | *Days* | *Days* | *No.* | *Inches* | *No.* | *Grams* |
| A×325 | Sprouted | 87.5 | 95.0 | 0.8 | −0.7 | 0.3 | *−1.9* | *−9* | −0.1 | −14.8 |
| A×164 | Dry | 95.0 | 100.0 | −.4 | *−3.0* | *−1.9* | *−1.8* | −7 | .0 | *−48.8* |
| A×164 [b] | do | 100.0 | 100.0 | *−1.0* | *−1.7* | *−1.6* | −.3 | *−9* | −.1 | −18.7 |
| A×164 | Sprouted | 92.5 | 100.0 | .0 | *−2.0* | *−1.2* | *−1.6* | *−5* | −.1 | *−44.5* |
| 420×164 | Dry | 72.5 | 95.0 | .0 | −.6 | −.4 | *−1.1* | −4 | −.6 | *−40.0* |
| 420×164 [b] | do | 95.0 | 97.5 | *−1.0* | *−2.1* | *−1.3* | −.3 | −3 | −.1 | −8.9 |
| 420×164 | Sprouted | 85.0 | 87.5 | .6 | .6 | .7 | *−1.0* | −3 | −.3 | −28.1 |
| 420×4–8 | do | 97.5 | 100.0 | .0 | *−1.0* | −.5 | *−1.2* | −3 | .0 | *−38.0* |
| 325×420 | do | 100.0 | 100.0 | −.1 | *−1.7* | −.9 | −.8 | −3 | .0 | *−30.1* |
| 325×420 [c] | do | 95.0 | 100.0 | .0 | *−2.7* | *−1.9* | *−1.0* | −3 | .0 | −1.9 |
| 540×164 | do | 95.0 | 100.0 | .6 | .1 | .2 | −.8 | −4 | −.1 | −23.9 |
| L×317 | do | 97.5 | 100.0 | −.5 | *−1.6* | −1.0 | −.7 | −3 | .0 | −10.3 |
| 317×461 | do | 100.0 | 97.5 | .4 | −.3 | −.2 | −.7 | 1 | −.3 | −23.7 |
| Gaspé | do | 95.0 | 100.0 | *1.5* | .7 | .6 | | | | |
| Do. [d] | do | 95.0 | 97.5 | .6 | −.3 | −.5 | | | | |

[a] Positive difference indicates iarovized lot exceeds check.
[b] Sprouted, not iarovized.
[c] Iarovized, continuous light.
[d] Iarovized at 38° F.

There appears to be no consistent relationship between the reduction in number of nodes and plant height as a result of the treatment. In some strains the iarovized plants exhibit a significant reduction in number of nodes and yet are not significantly shorter than their controls.

Where any differences exist in number of ears, the iarovized material always has the lower number. None of the differences, however, is significant.

All of the iarovized material exhibits a reduced yield of shelled grain per plant, only five of the comparisons, however, being significant. The reductions in yield indicated in table 2 as being significant represent a reduction of approximately 15 to 30 percent. It is worthy of mention that the comparison exhibiting the least reduction in yield was significantly earlier in pollen shedding and silking than its controls and was iarovized under continuous light.

In the variety Gaspé iarovization at 75° F. or at 38° F. for a 14-day period was ineffective in hastening sexual maturity.

### DARKNESS REQUIREMENT

According to the theory advanced by Lysenko (*4*), short-day plants require light for processes of growth and the absence of light (darkness) for the initiation of reproduction. The necessary darkness may be supplied continuously during the early stages of the plant's development or as periods alternating with light, as in day and night, during a greater portion of the plant's development. Iarovization carried on in the dark is presumed to be effective, in the case of short-day plants, because it satisfies the plant's requirement for

darkness. With this requirement satisfied, the plants are able to benefit from the long days, and hastened sexual maturity results.

The theory of a "darkness requirement" for the initiation of reproduction in corn, at least for some varieties of the temperate regions, is not in agreement with several facts. Corn has been grown in the greenhouse at the Arlington Farm during two winters under continuous illumination (normal day plus artificial illumination) without any material delay in the onset of flowering or maturity. In the winter of 1932–33 four inbred strains representing a considerable portion of the seasonal range of Corn Belt varieties were grown under continuous illumination from the dry seed to maturity. The plants were perfectly normal in their vegetative development and seasonal maturity.

As a further test for the necessity of a dark period, the same strains of corn were grown during the summer, one set of plants being exposed to the normal day, and a second set to continuous illumination (normal day plus artificial illumination). The results are presented in table 3.

TABLE 3.—*Effect of day length on sexual maturity in 4 inbred strains of corn*

| Inbred strain no. | Period from planting till pollen shedding when grown with a day length of— | | |
|---|---|---|---|
| | 12 hours | 16 hours | 24 hours |
| | *Days* | *Days* | *Days* |
| 616 | | 52 | 60 |
| A | | 55 | 61 |
| 228–4–8 | 61 | 62 | 69 |
| 461 | | 60 | 66 |

Continuous illumination resulted in approximately a week's delay in pollen shedding. For the one strain grown also with a 12-hour day, there was no significant difference in earliness between the 12-hour and 16-hour photoperiods.

The classification of corn as a short-day plant does not appear to be based on adequate experimental evidence. It is true that the work of Garner and Allard (*2*), Emerson (*1*), and McClelland (*5*) has shown that some varieties respond to a short day (10 to 12 hours of light). However, all of the varieties used by these investigators were tropical or semitropical sorts naturally adapted to short-day conditions. Varieties which are adapted to Corn Belt or more northern conditions and which normally bloom during a long day (15 to 18 hours of daylight) have not been adequately studied. In three strains grown in the greenhouse, augmenting the winter day (11 to 13 hours) by artificial illumination for 4.5 or 8.5 hours has not resulted in a significantly delayed sexual maturity.

Teosinte has been shown by Emerson (*1*) to respond to a short day. It was thought that a comparison of the effectiveness of darkness supplied to this plant during the iarovization process and as a daily photoperiod would be instructive. Three lots of teosinte were planted May 19, one of them having been iarovized. The iarovized lot and one of the others were exposed to the natural day. There

was no significant difference in sexual maturity of these two lots, both showing tassels September 16 and shedding pollen October 3. The second noniarovized lot was exposed to a 10-hour day. It showed tassels June 6 and shed pollen June 10, having been exposed to fewer hours of darkness (328) during this period than had the iarovized lot (336 hours) during the period of iarovization. It seems clear from this that length of day is much more important in determining the time of sexual maturity than is any darkness requirement that teosinte may possess (fig. 1).

FIGURE 1.—Response of teosinte to darkness. The plant on the right was exposed to darkness for a 14-hour period daily. The one on the left was exposed to darkness continuously for 14 days followed by exposure to darkness for an 8- to 9-hour period daily. Photographed June 19, 31 days after planting.

McKinney and Sando (*8*) have shown that after the iarovization treatment the attainment of sexual maturity in winter wheat is greatly influenced by day length. To determine whether a similar condition exists in short-day crops, a second set of material, common millet and the corn hybrid A×164, were iarovized at 80° F. at the moisture contents and for the periods recommended, one lot of seed of each crop in continuous darkness and a duplicate lot in continuous light (normal day plus artificial illumination). Following iarovization, the various lots, including dry and germinated checks, were grown in pots under a 16-hour and a 24-hour day.

The treatment given the millet was ineffective in hastening sexual maturity under either light treatment. The corn plants receiving the 16-hour day responded approximately as they had done in the field. For the plants grown under continuous illumination, iarovization with continuous light was superior to iarovization with continuous darkness in promoting early flowering, and both lots from iarovized seed were earlier than those from either the germinated or dry checks. The results are presented in table 4. There is nothing in the results obtained in these experiments with corn and teosinte to lend support to the darkness-requirement theory of Lysenko.

TABLE 4.—*Influence of day length on the attainment of sexual maturity in corn*

| Photoperiod (hours) | Period required to attain sexual maturity under indicated treatments | | | |
|---|---|---|---|---|
| | Iarovized | | Control | |
| | Continuous dark | Continuous light | Germinated | Dry |
| | *Days* | *Days* | *Days* | *Days* |
| 16 | 64 | 61 | 65 | 67 |
| 24 | 76 | 71 | 80 | 81 |

## DISCUSSION

The iarovization of certain corn hybrids resulted in a statistically significant hastening of sexual maturity. The difference, however, was so slight as to be of no importance agronomically. The general reduction in germination and vigor associated with the iarovization treatment appears to be a serious limitation of the method. In this respect these results depart rather drastically from those reported by the Russian investigators. The reason for the failure of agreement with their results is not entirely clear.

The Russians emphasize the fact that varieties differ markedly in their iarovization requirements. It is possible that all of the strains used in these tests require special conditions during iarovization, though this does not seem likely.

The experiments of McKinney and Sando (*8*) and the results reported here (table 4) are in agreement in indicating that the day length following iarovization has an important effect on the reaction. The plants in the present studies (tables 1 and 2) were grown under a daily photoperiod of approximately 15 hours, and those in the Russian work presumably under a longer daily photoperiod. It seems probable that at least part of the difference between the results in the two places may be ascribed to the day length under which the plants were grown. The claims for the necessity of darkness during the iarovization process, however, are not substantiated by either the field or greenhouse tests.

## SUMMARY

Iarovization of corn, as practiced, consistently reduced the percentage of germination. It also resulted in a general reduction in the number of visible nodes, in plant height, number of ears, and weight of shelled grain per plant.

Pollen shedding and silking were significantly accelerated by iarovization in some strains but not to an extent to be of any agronomic importance.

There was no evidence of a darkness requirement for corn. Several strains of corn were grown to maturity under continuous light. Iarovization in continuous light was just as effective as that in continuous darkness.

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# THE MICROBIAL DECOMPOSITION OF SUCCESSIVE CUTTINGS OF ALFALFA HAY UNDER AEROBIC CONDITIONS[1]

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## INTRODUCTION

The present investigation was undertaken to determine whether there is any difference in the decomposition of successive cuttings of alfalfa hay aerobically fermented by soil micro-organisms.

A review of the literature gives little information regarding the influence of chemical composition on the microbial decomposition of successive cuttings of alfalfa hay.

Falck and Haag (*1*)[2] concluded from their studies that in the microbiological decomposition of plant materials two distinct processes take place, namely, destruction and corrosion. The effect of destruction is to decompose the cellulose and pentosans, the lignins being very little affected. Corrosion, on the other hand, causes slow decomposition of both lignin and cellulose.

Starkey (*3, pp. 293–294*) stated:

> When the decomposition of organic matter is measured by the amount of $CO_2$ produced, it should be kept in mind that the $CO_2$ is not the only product formed from the carbon of the organic matter * * * since various intermediate products may be formed * * *. In general, however, the incomplete decomposition products of some organisms are further attacked by others and sooner or later appear as $CO_2$. * * * The $CO_2$ produced from soils should, therefore, give a reliable although not an absolute index of the decomposition of organic matter.

Waksman and Tenney (*6*) found that when a comparison is made of the rapidity of decomposition of a plant which has been harvested at different stages of growth, different results are obtained. The more mature the plant is the less readily does it decompose. Their analysis showed that a third of all the constituents of the young rye plant, on a dry basis, consisted of water-soluble substances, including considerable quantities of sugars and amino acids. The young plant contained, on a dry basis, 2.5 percent of nitrogen, 7.7 of ash, 16.6 of pentosan, 18.06 of cellulose, and 9.9 percent of lignin. With the advance in the age of the plant, there was a rapid decrease in the nitrogen and in the fat and ash content and a gradual increase in the cellulose, pentosan, and lignin content. There was considerable decrease in the amount of water-soluble constituents. The mature plant (exclusive of the grain and roots) contained 0.24 percent of nitrogen, 22.9 percent of pentosan, 36.3 percent of cellulose, 19.8 percent of lignin, and 9.9 percent of water-soluble substances.

## EXPERIMENTAL METHODS

In order to determine differences in the microbial decomposition of successive cuttings of alfalfa hay, two experiments, each in duplicate, were conducted. Three cuttings of hay were collected from each of

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[2] Reference is made by number (italic) to Literature Cited, p. 1126.

the 1931 and 1932 crops under similar conditions but from different fields. The hay used in the first experiment (1931 crop) was collected from a field on the United States Department of Agriculture Experiment Farm at Beltsville, Md., on which heavy manuring and intensive cultivation methods were used. The hay used in the second experiment (1932 crop) was collected from a privately owned field on which only ordinary fertilizing and cultivation methods were used. As Van der Spuy and Stead (*4*) have shown by analyses of plants at various stages of growth that the nutritive value of lucerne hay is highest when the hay is cut at the 10-percent stage of flowering, and also that the highest yield of hay is obtained by cutting at this stage of growth, the alfalfa hay for each experiment was collected when most of the plants were in partial bloom. Each cutting was obtained from the

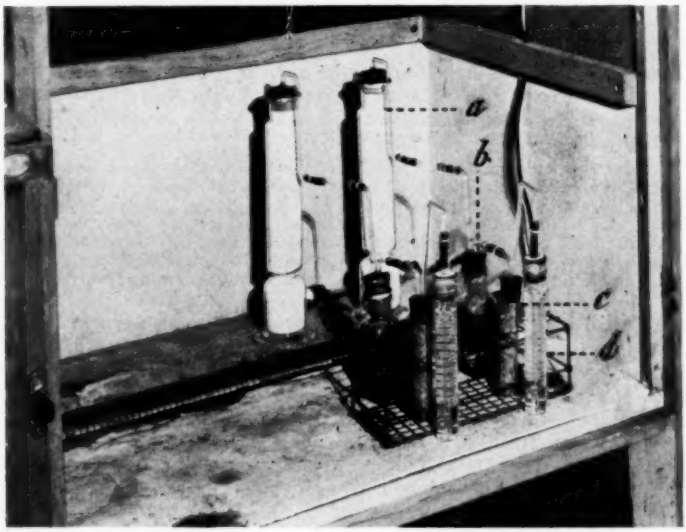


FIGURE 1—Aerating apparatus for the determination of carbon dioxide in fermenting plant materials, *a*, Soda lime tower; *b*, humidifier; *c*, fermentation tube; *d*, spiral absorber.

same area in each field in order that environmental growth factors would be the same. Immediately after cutting, the material was sun-dried for several days and then passed through a grinder.

In the first experiment, two 10-gram samples (calculated on a dry basis) of each of the three cuttings were inoculated with sufficient soil infusion to give a total moisture content of 43 percent. In the second experiment, two 6.5-gram samples of each cutting were similarly prepared. The soil infusion used in each experiment was prepared by mixing 100 grams of soil, obtained from the same area in the field from which each cutting of hay was obtained, with 150 cubic centimeters of water and filtering through sterile cotton. The filtrate was thoroughly shaken and used as the inoculum for each of the three cuttings of hay.

The inoculated hay samples were placed in large test tubes fitted with aeration tubes extending to the bottoms, connected to an aerat-

ing apparatus, and incubated at 30° C. for 30 days. Each aerating apparatus (fig. 1) consisted of a soda lime tower, *a*, containing wet pieces of sponge for moistening the air before it was passed through the hay sample; a fermentation tube, *c*, containing the hay sample; and a spiral absorbing tube, *d*, containing potassium hydroxide (*2*) to absorb the carbon dioxide produced from the fermenting hay.

Air was passed through the apparatus from a pressure line connected to a large "bleeder" bottle and then to the soda lime tower. The "bleeder" bottle was necessary to reduce the pressure and also to adjust the air flow at any desired rate. It consisted of a 15-liter bottle equipped with inlet and outlet tubes and a stopcock through which the excess pressure was released. The air flow was regulated at such a rate that the gas bubbles passing through the spiral tube containing the potassium hydroxide came in contact with the absorbing liquid long enough to allow for complete absorption. This rate of flow was found to be approximately 2 liters of air per hour. Determinations were made to check the efficiency of the soda lime towers and spiral absorbers. Daily titrations of the potassium hydroxide were made, and the quantities of carbon dioxide evolved from the fermenting hay were determined and recorded as milligrams of carbon.

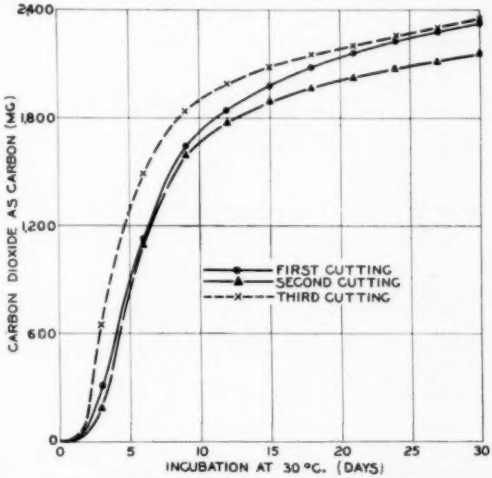


FIGURE 2.—Total carbon dioxide evolved during the microbial decomposition of three cuttings of alfalfa hay from the 1931 crop.

Oven-dried samples from the three cuttings of the 1931 crop were analyzed for the important chemical fractions by the modified method of Waksman and Tenney (*7*). Owing to limited facilities, the hemicelluloses, cellulose, and lignin were not directly determined but were recorded as the fraction soluble in 2-percent HCl, the fraction soluble in 80-percent $H_2SO_4$, and the residue, respectively. At the end of the fermentation period, the material remaining was dried to constant weight at 105° C. From the results obtained by the difference in weight before and after fermentation the percentage loss due to microbial action was determined. The oven-dried material was then analyzed as previously mentioned. Each determination was calculated on the basis of the original weight of material used, thereby showing the actual percentage loss of each fracton caused by the microbial decomposition. The chemical analyses on both the unfermented and fermented hays were repeated several times in duplicate.

## RESULTS

Results on the total quantities of carbon dioxide evolved during the microbial decomposition of three cuttings of alfalfa hay from the 1931 and 1932 crops are shown in figures 2 and 3. In both experiments (figs. 2 and 3), the third cutting showed the greatest decomposition, the first cutting was next in order, and the second cutting showed the least decomposition. Results on the daily titrations of carbon dioxide evolved during the microbial decomposition of three cuttings of alfalfa hay from the 1931 crop are shown in figure 4.

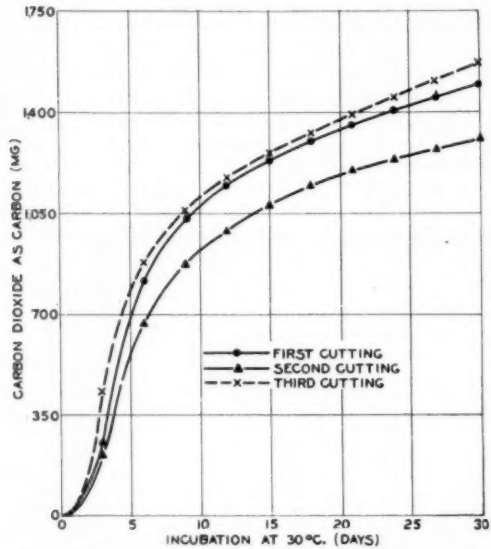


FIGURE 3.—Total carbon dioxide evolved during the microbial decomposition of three cuttings of alfalfa hay from the 1932 crop.

It will be noted that the greatest variation in the rate of decomposition of the three cuttings of hay occurred during the first 10 to 12 days of the fermentation, after which it gradually decreased to the end of the test period. Similar results were noted in the second experiment (1932 crop). At the end of the first experiment, the percentage loss of dry matter due to microbial decomposition was 41.3 for the third cutting, 39.2 for the first, and 35.8 for the second. In the second experiment, the percentage loss was 43.6 for the third cutting, 41.4 for the first, and 37.3 for the second.

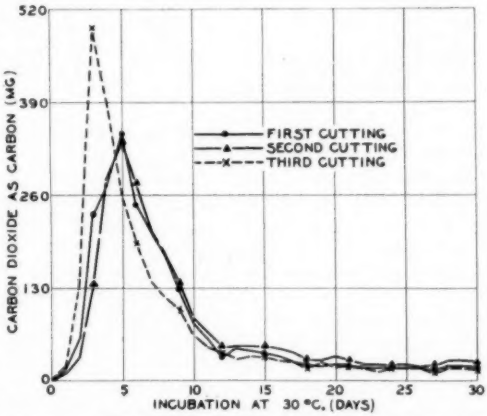


FIGURE 4.—Daily quantities of carbon dioxide evolved during the microbial decomposition of three cuttings of alfalfa hay from the 1931 crop.

Chemical analyses were made on oven-dried samples from the three cuttings of hay used in the first experiment before and after microbial decomposition. The results shown in table 1 were included to give the approximate chemical

composition of each cutting of hay before fermentation and similar data after fermentation, showing the percent loss in each of the fractions. It is noted that in the larger fractions (cold water, 2-percent HCl, and 80-percent $H_2SO_4$), although the percentages in the original hays were approximately the same in each cutting, the percentage losses after fermentation varied. According to Waksman and Tenney (*5*), the various fractions were composed of the following constituents: (1) The ether fraction contained fats and waxes; (2) the cold-water fraction contained simple carbohydrates, various amino acids, peptides, and soluble minerals; (3) the hot-water fraction contained starches, pectins, certain hexosans, and various nitrogenous compounds; (4) the 2-percent HCl fraction contained hemicelluloses and protein; (5) the 80-percent $H_2SO_4$ fraction contained cellulose and protein; and (6) the residue contained lignin, protein, and ash.

TABLE 1.—*Percentage composition (by fractions containing various constituents) of successive cuttings of alfalfa hay from the 1931 crop before and after microbial decomposition, and percentage loss of each fraction*

[On moisture-free basis]

| Chemical fraction | First cutting | | | Second cutting | | | Third cutting | | |
|---|---|---|---|---|---|---|---|---|---|
| | Original material | After 30 days' decomposition | | Original material | After 30 days' decomposition | | Original material | After 30 days' decomposition | |
| | | Calculated on original weight | Loss | | Calculated on original weight | Loss | | Calculated on original weight | Loss |
| Ether | 2.0 | 1.7 | 15.0 | 3.0 | 2.7 | 10.0 | 2.2 | 1.7 | 22.7 |
| Cold-water | 24.6 | 10.3 | 58.1 | 24.2 | 13.7 | 43.3 | 26.7 | 13.2 | 50.5 |
| Hot-water | 5.1 | 3.2 | 37.2 | 4.2 | 4.0 | 4.7 | 4.6 | 2.8 | 39.1 |
| 2 percent HCl | 28.6 | 17.3 | 39.5 | 29.2 | 15.8 | 45.8 | 30.3 | 17.4 | 42.5 |
| 80 percent $H_2SO_4$ | 23.3 | 15.4 | 33.9 | 22.2 | 14.0 | 36.9 | 19.4 | 9.8 | 49.4 |
| Residue | 14.0 | 11.0 | 21.4 | 13.6 | 11.9 | 12.5 | 13.3 | 12.0 | 9.7 |
| Protein, insoluble in cold $H_2O$ [a] | 12.3 | 7.3 | 40.6 | 13.2 | 7.2 | 45.4 | 15.5 | 8.6 | 44.5 |
| Total nitrogen [a] | 2.5 | 1.5 | 40.0 | 3.1 | 1.6 | 48.3 | 3.1 | 1.8 | 41.9 |
| Ash [a] | 9.7 | 9.7 | 0 | 9.7 | 9.7 | 0 | 9.6 | 9.6 | 0 |

[a] Analyses carried out on separate samples.

Determination of the quantity of the individual constituents in each fraction was not made. In the larger fractions the variations in the losses the three cuttings were probably due to differences in the amounts of easily fermentable substances.

The types of micro-organisms in the fermenting hay greatly influenced the decompositions. The ground hay was very porous, hence it favored the rapid development of fungi, especially actinomycetes, which are capable of rapidly decomposing organic matter. Slimy masses of bacteria were also present in the fermenting hay although a more compact medium would have favored their development. The microbial decompositions were probably accelerated by the high nitrogen content in each cutting.

## SUMMARY

A study was made of the influence of chemical composition on the microbial decomposition of successive cuttings of alfalfa hay. Data on the microbial decomposition of each cutting were obtained by measuring the daily evolution of carbon dioxide during a test period of 30 days, and by chemical analyses before and after fermentation.

Of the three cuttings of hay tested from the 1931 and 1932 crops, the third cutting underwent the greatest decomposition, the first cutting was next in order, and the second cutting showed the least decomposition.

The greatest variation in the rate of decomposition occurred during the first 10 to 12 days of the fermentations, after which it gradually decreased to the end of the experiment.

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# PRESS FLUID FROM HEATED BEEF MUSCLE[1]

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## INTRODUCTION

Juiciness, which is dependent upon the fluid content of muscle, is an important factor in palatability of meat, and quality is closely associated with palatability. Howe [2] describes juiciness in meat as its readily expressible liquid.

A grading chart with descriptive terms for scoring factors of palatability in meat has been developed by the cooking committee of the cooperative meat investigations committee.[3] This chart grades quantity of juice in meat as "very juicy", "moderately juicy", "slightly dry", "dry", "very dry", and "extremely dry." Quality of juice is graded as "very rich", "rich", "moderately rich", "slightly rich", "perceptible", "slightly perceptible." Grading the quantity and quality of juice in meat by such a method depends upon individual differences and standards of the judge. More accurate methods of determining both quantity and quality of juice are necessary for scientific experimentation in order that data of different investigators may be definitely compared.

The literature describes no definite method for pressing the fluid content from muscle, and little data can be found dealing with this pressed fluid.

This investigation was undertaken to develop a laboratory method for pressing out muscle fluid, by which different meat samples might be given a relative rating for quantity of press fluid or juice.

The purposes of this study were (1) to develop an apparatus for the removal of press fluid from roasted beef muscle; (2) to standardize methods for determining the percentage of press fluid and the ratio between press fluid and dry matter, and for obtaining press fluid for chemical analysis; (3) to use the standardized methods for studying press fluid in two beef muscles, psoas major, and biceps femoris; and (4) to compare the moisture, ether extract, and nitrogen contents of the press fluid from roasted beef muscle under pressure for two periods of 5 minutes and 20 minutes.

## APPARATUS AND MATERIALS

The apparatus used in this study is called a "pressometer" (fig. 1). The term "press fluid" is used in preference to "juice" since in meat studies juiciness is graded by the individual's reaction when meat is eaten and probably includes not only fluids which are present in meat, but depends also upon the flow of saliva stimu-

[1] Received for publication Jan. 18, 1934; issued July 1934. Contribution from Minnesota Agricultural Experiment Station, Scientific Journal Series, Paper No. 1246. The authors gratefully acknowledge the technical assistance of Christian Dane and George Steinacher.

[2] HOWE, P. E. RELATION OF COOKING TO THE STUDY OF THE QUALITY AND PALATABILITY OF MEAT. Jour. Home Econ. 19: 8-15. 1927.

[3] ALEXANDER, L. M., CLARK, N. G., and HOWE, P. E. METHODS OF COOKING AND TESTING MEAT FOR PALATABILITY. Revised February 1933. Supplement to National Project Cooperative Meat Investigations. U.S. Dept. Agr., Bur. Home Econ. and Bur. Anim. Indus. 36 pp., illus. 1933. [Mimeographed.]

lated by meat extractives. Therefore, "press fluid" is used to designate the fluid consisting of moisture plus the soluble material plus the colloidal fraction that is pressed from muscle by the pressometer.

The pressometer (fig. 1) consists of a heavy cast-iron base with an attached motor. The muscle sample is wrapped in filter cloth (fig. 3) and placed in a brass tray (fig. 2) which is fitted into a grooved platform in the apparatus. Starting the motor, the platform with

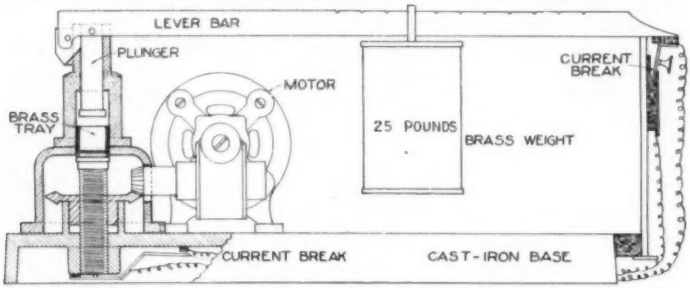


FIGURE 1.—Cross section of the pressometer, giving detail of working mechanism.

tray is driven upward by means of a large screw until it reaches a stationary plunger, the bottom of its base having the same dimensions as the inside of the tray. A 25-pound brass weight, which is suspended from the lever bar, exerts pressure on the sample. The pressure may be varied from 250 to 500 pounds by placing the weight at different points on the lever. When the desired pressure has been attained, the lever arm is lifted, breaking the current, and automatically arresting an increase in pressure. The actual time of pressure is checked by an automatic timer or stop watch. To carry the platform downward, the switch is reversed.

Samples were taken from beef roasts averaging 1½ pounds, from the left and right sides of the same animal. The roasts were stored in an electric refrigerator at 4° to 6° C. until used for cooking.

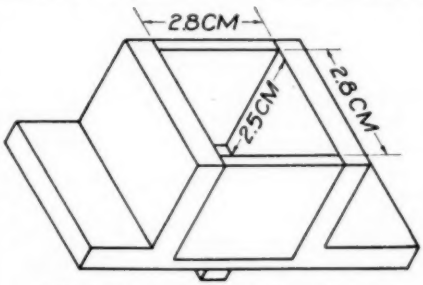


FIGURE 2.—Brass tray for holding muscle sample.

The rump was removed from the wholesale round by cutting parallel and ventral to the pelvic bone (aitch bone). On the medial side (inside or top round) at this point, there are two large muscles, the posterior one being the semimembranosus muscle, immediately in front of which lies the adductor muscle from which samples were taken for the removal of press fluid.

Roasts were prepared according to the method given in the quality tests officially accepted by the cooking committee of the cooperative meat investigation committee,[4] but were not seared. The exterior

[4] ALEXANDER, L. M., CLARK, N. G., and HOWE, P. E. See footnote 3.

fat was removed, and the meat wiped with a damp cloth; it was then weighed, and all data for computing losses were recorded. The roast was tied with a string so that it was cylindrical in shape. The center of the bulb of a weighed, straight thermometer was inserted in the center of the roast. The meat was placed on a heavy wire rack, the rack being 1.25 cm from the bottom of the pan, in a weighed sheet-iron roasting pan (23.8 by 18.4 by 6 cm). The pan was set crosswise in the oven which was preheated to 125° C. The meat was roasted until the thermometer registered 60°.

## STANDARDIZATION OF METHOD FOR USING PRESSOMETER

The first work on pressing muscle was done by placing the meat in a tray (2.5 by 2.7 by 0.8 cm) with a sieve bottom, the press fluid being collected in a tray placed directly under it. Because of the small size of the sample with which it was necessary to work, much of the fluid was lost either by evaporation or by adherence to to the tray, and small quantities of the solid part of the muscle were pressed through the sieve. Later, a tray with a slight metal elevation (height 0.3 cm) in the center was used, thus forming a groove around the edge, where the fluid could collect. This method, also, proved to be inadequate since it was difficult to press all of the fluid from the edges of the muscle and to remove it from the tray; therefore, it was decided to wrap the sample in a piece of cloth.

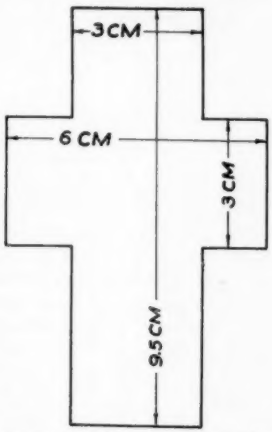

FIGURE 3.—Cloth cross for wrapping muscle sample.

After trying different kinds, sizes, and shapes of cloth for wrapping the muscle sample, it was decided that unsized filter cloth, cut cross shape (fig. 3), gave the best results. The crosses were cut from the filter cloth after it had been boiled 10 minutes in distilled water and then dried.

For sampling, a slice of meat was cut from the center of the roast with a sharp thin-bladed knife. A mechanical gage was used for determining the exact thickness. In order to obtain the most desirable thickness for cutting the meat, slices of different thicknesses (2.5, 1.87, and 1.25 cm) were tried. Those cut 2.5 cm thick were too large and showed that all the press fluid was not absorbed by the cloth. The press fluid from the 1.87-cm and 1.25-cm slices was absorbed by the cloth. It was decided to use the 1.87-cm slice in order to obtain more easily a sufficient quantity of fluid for chemical analysis.

A brief study was made of metal borers of different sizes and shapes. Two round borers (one, 1.27 cm in diameter, and the other, 2.4 cm in diameter) and a square borer (1 cm square) were used. The round borers were more easily used than the square one. The round borer 1.27 cm (fig. 4) in diameter was chosen, as the small sample had a more uniform structure of muscle fibers than the larger one.

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Experimental work was carried out using different pressures for 10 minutes. The average [5] of press fluid pressed out by 112 pounds pressure was 43.90 percent; by 168 pounds pressure, 49.73; by 226.5 pounds pressure, 50.26; by 250 pounds pressure, 56.84; and by 500 pounds pressure, 58.61 percent. The data obtained showed that increased pressure yields more press fluid. Two hundred and fifty pounds pressure was selected because it was found that this pressure could be used also with chicken muscle, which is short fibered. Some of the solids of this tender muscle were pressed into the cloth when 500 pounds pressure was used.

To determine the optimum length of time for the removal of press fluid, four periods were used: 5, 10, 15, and 20 minutes. The mean press fluid for seven samples was as follows: 5 minutes, 49.00 percent; 10 minutes, 49.28 percent; 15 minutes, 50.64 percent; and 20 minutes, 50.66 percent.

Since there was not a great variation in the mean percentage of press fluid obtained from these different periods, the 10-minute period was used, as it allowed sufficient time for weighing samples and less time for evaporation.

FIGURE 4.—Borer, 7.6 cm in length and 1.27 cm in diameter, used for cutting muscle sample.

The average press fluid in nine muscle samples was 49.93 percent, based on the difference between the weight of the original sample and the pressed muscle. The average press fluid in the cloth from the same samples was 48.98 percent, based on the weight of the original dry cloth and the cloth moistened with the pressed fluid. The slightly lower percentage of fluid in the cloth than in the muscle was probably due to a faster evaporation in the cloth.

To compare the quantity of press fluid from roasts of different temperature, samples were cut (1) at the time the roasts were taken from the oven, (2) 2 hours after taking from the oven, (3) 3½ hours after taking from the oven. The mean press fluid from roasts at approximately 55.5° C. was 49.64 percent; at 23.3°, 49.74 percent; and at 21.1°, 49.52 percent. From the mean percentages of press fluid obtained from samples of roasts at these different temperatures, it can be seen that there was not an appreciable difference in the total quantity. It seemed evident that all of the small samples of muscle were about the same temperature at the actual time of pressing. To prove this, thermocouples were used to obtain the temperature of the small samples from the roasts of varying temperatures, and it was found that by the time the sample was cut, weighed, and wrapped in cloth, the muscle had almost reached room temperature (21°). Since there was so little difference in the quantity of press fluid, all roasts were cooled to 40° before sampling.

## METHODS SELECTED FOR OBTAINING PRESS FLUID FROM ROASTED BEEF MUSCLE

From the preliminary work the following were chosen as desirable methods for obtaining press fluid from roasted beef muscle and were used in succeeding experiments.

[5] The percentages press fluid for 112, 168, and 227.5 pounds pressure were obtained by using an unimproved pressometer.

A slice 1.87 cm thick was cut from the center of the roast by means of a sharp thin-bladed knife, using a mechanical gage for determining the thickness of the slice. Three adjacent samples were cut from the center of the slice with a round borer 1.27 cm in diameter (fig. 4). Each sample was transferred to a numbered, previously weighed, aluminum dish containing a piece of dry, weighed, unsized, shrunken filter cloth that was cut cross shape. The dish with cloth and muscle sample was then weighed. The sample was carefully wrapped and placed in the tray (fig. 2), which was inserted in the pressometer and allowed to remain for 10 minutes at a pressure of 250 pounds. The muscle sample and the cloth were removed from the tray and placed in separate, dried, weighed aluminum dishes and weighed; samples were kept in the desiccator until ready for weighing. Rapid work was necessary from the time the sample was cut to the last weighing, so as to avoid evaporation losses. Forceps were used for all handling.

The percentage of press fluid in the muscle was found by dividing the weight of the press fluid by the weight of the muscle sample before pressing. The weight of the press fluid was found by subtracting the weight of the pressed muscle sample from the weight of the unpressed sample.

## METHOD USED IN OBTAINING RATIO OF PRESS FLUID TO DRY MATTER FROM HEATED MUSCLE

The method for obtaining the quantity of press fluid from heated muscle, as previously explained, was followed. The two aluminum dishes with weighed samples of pressed muscle in one and the cloth containing the press fluid in the other were then dried in a Freas vacuum oven at 65° C. The ratio of press fluid to dry matter was calculated by dividing the weight of the press fluid by the weight of the dry matter. The dry matter includes both that in the residual muscle and that adhering to the cloth.

## METHOD USED FOR OBTAINING PRESS FLUID FOR CHEMICAL ANALYSIS

For chemical analysis of the press fluid, samples of muscle were cut from the center slices of the roast.

The sample was set in the center of a weighed, cross-shaped filter cloth, wrapped, and placed in the pressometer and allowed to remain for 5 minutes at a pressure of 250 pounds; the pressed muscle was then discarded and the process was repeated, using the same piece of cloth with two other muscle samples. The cloth was removed to a weighed corked bottle and placed in the desiccator. When four pieces of cloth were similarly filled with press fluid, the bottle and cloth were weighed and the amount of press fluid was calculated. This quantity of press fluid (5½ g or more) was sufficient to determine the ether extract, nitrogen, and moisture content. The corked sample bottle, containing the filter cloth with pressed fluid, was kept in a cold room (−14° to −15° C.) until needed for analysis.

The sample, after being prepared for analysis, was cut in pieces approximately one-fourth inch square and was divided into five aliquot portions, each being placed in a weighed aluminum dish. The covered dishes were allowed to stand at room temperature for

15 minutes, then weighed. Moisture, ether extract, and nitrogen were determined by the official methods of analysis of the Association of Official Agricultural Chemists.[5]

## EXPERIMENTAL DATA FROM USE OF THE PRESSOMETER

The three methods of experimentation were used for comparing (1) the press fluid in two beef muscles, one tender (psoas major) and one less tender (biceps femoris), and (2) the moisture, ether extract, and nitrogen in the press fluid from roasted adductor muscle when the samples were kept under pressure for two periods, 5 minutes and 20 minutes.

### RATIO OF PRESS FLUID TO DRY MATTER IN ROASTED BEEF

Table 1 presents the mean grams of fluid per gram of dry matter from a tender muscle (the psoas major) of roast beef, and a less tender muscle (the biceps femoris). The mean grams of fluid per gram of dry matter (1.847 for biceps femoris and 1.823 for psoas major) did not vary significantly, as determined by means of the $t$ test [6]; $t$ is 0.226 and $P$ is between 0.90 and 0.80 which means that in 80 to 90 cases out of 100 this difference is due to chance.

TABLE 1.—*Mean* [a] *grams of press fluid per gram of dry matter in the muscles, biceps femoris and psoas major, from roasted beef*

| Roast no. | Biceps femoris muscle | Roast no. | Psoas major muscle |
|---|---|---|---|
| 1 | 1.620 | 9 | 1.815 |
| 2 | 2.076 | 10 | 1.660 |
| 3 | 1.583 | 11 | 1.336 |
| 4 | 1.946 | 12 | 2.160 |
| 5 | 1.903 | 13 | 2.013 |
| 6 | 1.796 | 14 | 1.596 |
| 7 | 2.100 | 15 | 1.926 |
| 8 | 1.753 | 16 | 2.080 |
| Mean | 1.847 | Mean | 1.823 |

[a] From 3 samples from center slice of roast.

### MOISTURE, ETHER EXTRACT, AND NITROGEN IN PRESS FLUID FROM ROASTED BEEF WHEN SAMPLES WERE UNDER PRESSURE FOR PERIODS OF 5 AND 20 MINUTES

The percentage of moisture in press fluid from the roasted adductor muscle of beef under pressure for periods of 5 and 20 minutes is given in table 2.

The mean percentage of moisture in the press fluid when the samples remained under pressure for 5 minutes was 89.974, and for 20 minutes 88.757, showing slightly less moisture after it had been under pressure for 20 than for 5 minutes. This slight difference may be due to greater evaporation when the sample remained under pressure for the longer time; $t$ is 1.640 and $P$ is between 0.20 and 0.10, which means that in 10 to 20 cases out of 100 this difference is due to chance. These data indicate that duration of pressing is not likely to affect the percentage of moisture in press fluid.

[5] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. Compiled by the committee on editing methods of analysis . . . Ed. 3, 593 pp., illus. Washington, D.C., 1930.

[6] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 4, rev. and enl., 307 pp., illus. Edinburgh and London. 1932.

TABLE 2.—*Percentage of moisture, ether extract, and nitrogen in press fluid from roasted adductor beef muscle under pressure for 5-minute and for 20-minute periods*

MOISTURE

| 5 minutes | | 20 minutes | |
|---|---|---|---|
| Roast no. | Moisture | Roast no. | Moisture |
| 18 | 90.96 | 19 | 88.46 |
| 20 | 89.37 | 21 | 89.52 |
| 22 | 90.32 | 23 | 89.05 |
| 24 | 90.44 | 25 | 90.67 |
| 26 | 90.39 | 27 | 90.72 |
| 28 | 89.28 | 29 | 90.54 |
| 30 | 89.53 | 31 | 87.94 |
| 32 | 91.15 | 33 | 90.38 |
| 34 | 90.49 | 35 | 88.52 |
| 36 | 86.80 | 37 | 90.01 |
| 38 | 90.71 | 39 | 91.08 |
| 40 | 89.24 | 41 | 86.49 |
| 42 | 82.25 | 43 | 78.83 |
| 44 | 89.71 | 45 | 90.38 |
| Mean | 89.974 | Mean | 88.757 |

ETHER EXTRACT

| | | | |
|---|---|---|---|
| 18 | 1.81 | 19 | 3.14 |
| 20 | 2.39 | 21 | 2.82 |
| 22 | 2.53 | 23 | 2.86 |
| 24 | 2.00 | 25 | 2.48 |
| 26 | 1.05 | 27 | 2.16 |
| 28 | 3.65 | 29 | 3.07 |
| 30 | 2.87 | 31 | 1.87 |
| 32 | 1.61 | 33 | .97 |
| 34 | 1.70 | 35 | 1.86 |
| 36 | 3.24 | 37 | 2.61 |
| 38 | 1.74 | 39 | .73 |
| 40 | 2.82 | 41 | 7.18 |
| 42 | 7.32 | 43 | 7.07 |
| 44 | 1.26 | 45 | 1.87 |
| Mean | 2.571 | Mean | 2.906 |

NITROGEN

| | | | |
|---|---|---|---|
| 18 | 1.040 | 19 | 1.020 |
| 20 | .980 | 21 | .950 |
| 22 | .984 | 23 | .965 |
| 24 | 1.021 | 25 | .946 |
| 26 | .950 | 27 | .927 |
| 28 | .938 | 29 | .942 |
| 30 | .997 | 31 | .985 |
| 32 | .975 | 33 | .906 |
| 34 | 1.005 | 35 | .925 |
| 36 | 1.002 | 37 | .950 |
| 38 | .888 | 39 | .866 |
| 40 | 1.000 | 41 | .957 |
| 42 | .963 | 43 | .964 |
| 44 | 1.010 | 45 | .954 |
| Mean | .982 | Mean | .947 |

The quantity of fat in press fluid may be an indication of the quality of press fluid or juice in meat. In order to determine whether time of pressing had any effect on the quantity of fat, the ether extract obtained from press fluid of roasted adductor beef muscle under pressure for 5 minutes was compared with that obtained under 20 minutes' pressure (table 2). In the case of ether extract, the 5-minute period gave a mean percentage of 2.571, and the 20-minute period one of 2.906, $t$ being 0.914 and $P$ between 0.40 and 0.30,

indicating that the difference is not significant.[7] A longer time under pressure is not likely to influence the final percentage of ether extract obtained from the press fluid, since muscle fat solidifies when cool.

The mean percentage of nitrogen (table 2) in the press fluid from the roasted muscle was found to be significantly higher after a 5-minute expression period than after a 20-minute period, being 0.982 percent for the former and 0.947 percent for the latter. The $t$ test applied to this difference gave $t=4.843$, and $P$ is less than 0.01. One might assume that more nitrogen would be obtained in press fluid when the longer period is used, because of the possible pressing out of colloidal material into the cloth. A possible explanation of the fact that the greater percentage of nitrogen appears during the 5-minute period than during the 20-minute period, is that the soluble nitrogen is pressed out at the beginning of the period. Gortner, Lawrence, and Harris [8] in their work on plant tissue, state that in some instances the fluid extracted by continuous pressing, without rearrangement of the tissue mass, may become less and less concentrated. This was found to be true in a series of extractions from cabbage leaves.

## SUMMARY

An apparatus called the "pressometer", developed to press fluid from heated beef muscle, is described.

Methods are explained for using the pressometer, for determining the percentage of press fluid and the ratio of press fluid to dry matter in heated beef muscle, and for obtaining press fluid from heated beef muscle for chemical analysis.

From this study the following observations may be made on the basis of statistical analysis:

The mean percentages of ether extract and moisture in the press fluid from the adductor muscle from roasted beef, did not vary significantly when samples were under pressure for 5 and for 20-minute periods.

The mean percentages of nitrogen in the press fluid from adductor muscle from roasted beef varied significantly when samples were under pressure for 5 and for 20-minute periods, the greater percentage of nitrogen appearing during the 5-minute period.

The mean percentages of press fluid from the muscles psoas major and biceps femoris from roasted beef did not vary significantly when the grams of press fluid per dram of dry matter were compared.

[7] Fisher, R. A. See footnote 6.

[8] Gortner, R. A., Lawrence J. C., and Harris, J. A. The extraction of sap from plant tissues by pressure. Biochem. Bull. 5: 139–142. 1916.

# CARBON DIOXIDE FORMATION BY CLEAN AND SCABBY POTATOES [1]

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## INTRODUCTION

Losses in weight of potato tubers in storage are due to both transpiration and respiration, but much the greater losses are due to the former. The checking of such losses by the tubers is largely through the skin. The skins of scabby potatoes are altered considerably by a parasitic growth which stimulates the formation of a loose and very permeable covering. Such tubers lose weight reapidly after removal from the soil. The gradual healing of the abnormal skin areas after the tubers have been in storage for some time tends to check these heavy initial losses and to restore the tubers almost to the same condition as healthy ones.

## PREVIOUS WORK

The relation of respiration to loss of weight in potatoes, apples, carrots, and other fruits and vegetables has been discussed in considerable detail by various authors. Especially important in this connection is the work of Appleman, Kimbrough, and Smith (*1*)[3] on the physiological shrinkage of potatoes in storage, and that of Kimbrough (*5*) on the respiration of potatoes during storage and transportation. Some interesting work on the internal gases of potato tubers has been reported by Magness (*7*). A detailed account of the relative losses in weight of clean and scabby tubers is given in an earlier report by the present writer (*6*).

The method used by all these investigators except the last mentioned is to pass carbon-dioxide-free air through a vessel containing a weighed lot of potatoes. This air sweeps out the carbon dioxide formed by the tubers. The air with its accumulated carbon dioxide is then passed through a solution such as barium hydroxide. A titration of this solution at the conclusion of the run gives the amount of carbon dioxide evolved.

The method used by the author (*6*) was the sampling method, the determination of the percentage of carbon dioxide in the 10-cc sample being determined by the Haldane apparatus. By this method the respiration of 10 pounds of potatoes kept under bell jars between October 4 and March 29 was only 2.471 for clean tubers or 3.757 for scabby ones. The temperature during much of this storage period was only 2° C. Although at the beginning of the season it has been 12°, it dropped rapidly to about 3° to 4°. The total respiration loss of the tubers kept by Kimbrough (*5*) is not given, but from his graph the

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[2] The writer is indebted to John B. Vander, assistant chemist, Vermont Agricultural Experiment Station, for the chemical work involved in the storage studies of the clean and scabby potatoes.

[3] Reference is made by number (italic) to Literature Cited, p. 1143.

loss between October 1 and February 7 (129 days) for a kilogram of tubers kept at 22° C. (71.6° F.) appears to be about 17. The respiration at 40° of Green Mountain tubers, according to Wright (*9*), was in milligrams per kilogram-hour on November 7, 5.11; on January 17, 3.57; and on March 24, 2.93. At these rates the total loss per kilogram would be for the 5 storage months, November to March, inclusive, about 15 g.

In a recent publication Smith (*8*) reviewed much of the literature and added extensive data on the effect of temperature, humidity, injury, depth of layer, etc., on potatoes in storage. Ordinary storage cellars were used in some of the experiments, and the loss in weight from respiration during a 7-month period ranged from 0.40 to 0.67 percent of the total weight of the tubers. The loss from respiration of mechanically injured tubers, while large at first, dropped rapidly until it was no greater than that of uninjured ones. The depth of the potatoes in the bin had little effect on the carbon dioxide formation, although it was somewhat higher in the bottom layers than in the top ones. The difference was noticeable only at the end of the storage season when sprouting was probably starting. Smith does not discuss the relative merits of the methods used for respiration determination.

## PURPOSE OF THE WORK

The purpose of the experiments here reported was primarily to ascertain the loss by respiration of scabby potatoes as compared with that of clean ones after various periods in storage, and to compare the results obtained by the aspirator method with those obtained by the sampling one.

## METHODS

Four lots of tubers as nearly alike as possible in size and number were placed under bell jars. Each lot weighed approximately 1,000 g. Two of the lots were composed of clean tubers and two of scabby tubers. The jars contained 8 liters of air after the tubers had been placed in them. To prevent the growth of mold, the tubers were immersed for 1 hour in a weak formaldehyde solution before they were placed in the jars, and fresh tubers were substituted if decay appeared. The jars were kept in a basement room the temperature of which ranged from 13° to 15° C.

The amount of carbon dioxide evolved was measured by (1) the aspirator method, and (2) the sampling or Haldane method. The amount of carbon dioxide formed in a jar of clean and in one of scabby tubers was determined by each method.

When the aspirator method was used laboratory air, freed from carbon dioxide and moisture (fig. 1), was passed through the bell jar containing the tubers and then into a small Erlenmeyer flask containing 100 cc of 0.1650 normal barium hydroxide solution. After the air in the bell jar had been passed for 24 hours through this solution, it was titrated with 2/10 N oxalic acid and the amount of carbon dioxide computed.

An analysis of the air inside bell jars containing clean or scabby tubers was made daily by the use of a Haldane apparatus during the early part of the storage season and usually at intervals of 2 days during the late fall. The tubers were left under the jars for periods of

5 to 7 days during the winter, the carbon dioxide being allowed to accumulate in the jar. The air in the bell jars was thoroughly mixed each time by the aid of a celluloid fan operated from outside to insure a homogeneous mixture.

The intercellular gas of the tubers used in the experiments was removed from a number of the lots. The method of extraction was

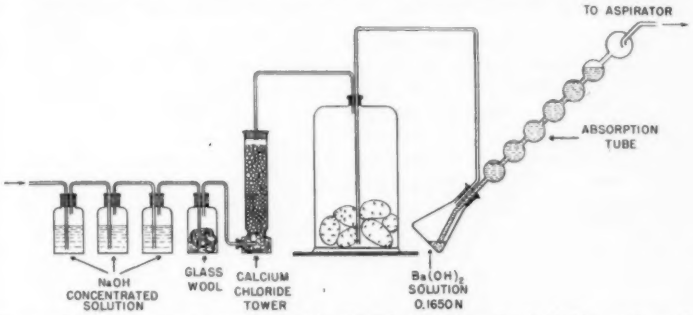


FIGURE 1.—Diagram of apparatus used for obtaining the carbon dioxide production by the use of a continuous current of carbon-dioxide-free air.

that described by Magness (7), and the determination of carbon dioxide and oxygen was made by a modified Henderson apparatus (fig. 2). The modification consisted in the use of a 3-cc calibrated tube instead of the usual 10-cc tube. Three cubic centimeters of gas was usually all that could readily be obtained. Eight plugs, 1 or 2 from each tuber, were placed in the mercury. The modified Henderson appa-

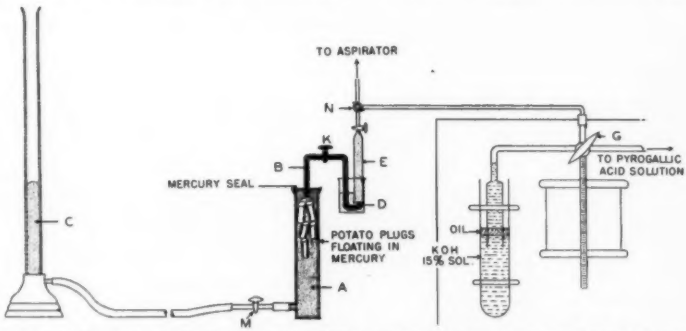


FIGURE 2.—Diagram of apparatus used for the extraction of internal gases from the potato tuber and for the determination of the percentage of carbon dioxide and oxygen in the 3-cc tube employed in the modified Henderson apparatus. *A*, Jar filled with mercury and potato plugs; *B*, tube filled with mercury; *C*, jar filled with mercury, connected by heavy rubber tubing with jar *A*, controlled by stop cock *M*, jar *C* can be raised and lowered; *D*, beaker filled with mercury in which is placed the gas-collecting tube *E*, controlled by stopcock *K*; *N*, stopcock from collection tube *E*, to aspirator or to $CO_2$ and $O_2$ apparatus; *G*, three-way stopcock to calibrated $CO_2$ apparatus immediately below or to pyrogallic acid solution on right but not shown in the figure.

ratus is not so accurate as the Haldane, but it is probably not more than 1 percent in error.

To determine the amount of carbon dioxide that would accumulate in air-tight potato bins, two large cylindrical, galvanized iron tanks, each 5½ feet high, were filled with clean or scabby potatoes. Before the potatoes were placed in the bins, glass tubes with a bore of about

2 mm (barometer) were placed in each so as to tap the air between the tubers at the 5-, 4-, 3-, 2-, and 1-foot levels. The upper end of each tube was closed by a pinchcock. Samples of the storage air at the various levels could be drawn through the tubes without disturbing the other air or the tubers.

## DATA

### LOSS OF CARBON DIOXIDE FROM CLEAN AND SCABBY TUBERS

Four lots of tubers were carried through the storage season under bell jars. These tubers were changed every 2 to 3 weeks from a supply kept under the same conditions. Two jars contained clean tubers and two contained scabby ones. The respiration in a clean and in a scabby jar was measured by the absorption-aspirator method and similar lots were measured by the sampling method. The experiments were continued from October 11, 1930, to March 19, 1931, a total storage period of 159 days. A summary of the results, presented in tables 1 and 2, shows that the general effect of scabbing was to increase the loss of carbon dioxide except during the last month or so of storage. The loss as determined by the absorption method was almost twice as great as that by the sampling method.

TABLE 1.—*Carbon dioxide production (milligrams) by clean and scabby potatoes during the storage season, as determined by the absorption and sampling methods*

| Month | Average production of $CO_2$ per kilogram-hour | | | | Average production of $CO_2$ per kilogram per day | | | |
|---|---|---|---|---|---|---|---|---|
| | Absorption method | | Sampling method | | Absorption method | | Sampling method | |
| | Clean potatoes | Scabby potatoes | Clean potatoes | Scabby potatoes | Clean potatoes | Scabby potatoes | Clean potatoes | Scabby potatoes |
| October | 8.8 | 10.0 | 6.7 | 8.7 | 211.2 | 240.0 | 160.8 | 208.8 |
| November | 4.1 | 6.0 | 2.7 | 4.0 | 98.4 | 144.0 | 64.8 | 96.0 |
| December | 3.3 | 5.9 | 1.6 | 2.2 | 79.2 | 141.6 | 38.4 | 52.8 |
| January | 4.2 | 4.9 | 2.1 | 2.4 | 100.8 | 117.6 | 50.4 | 57.6 |
| February | 5.5 | 5.1 | 2.7 | 2.8 | 132.0 | 122.4 | 64.8 | 67.2 |
| March | 9.3 | 6.7 | 4.0 | 3.5 | 223.2 | 160.8 | 96.0 | 84.0 |

TABLE 2.—*Total carbon dioxide production (grams per kilogram) by clean and scabby potatoes, by the month or part of the month, and also for the entire storage season, as determined by the absorption and sampling methods*

| Month | Absorption method | | Sampling method | |
|---|---|---|---|---|
| | Clean potatoes | Scabby potatoes | Clean potatoes | Scabby potatoes |
| October (20 days) | 4.224 | 4.800 | 3.216 | 4.176 |
| November | 2.952 | 4.320 | 1.944 | 2.880 |
| December | 2.455 | 4.390 | 1.190 | 1.637 |
| January | 3.125 | 3.645 | 1.562 | 1.785 |
| February | 3.696 | 3.427 | 1.814 | 1.881 |
| March (19 days) | 4.241 | 3.055 | 1.824 | 1.596 |
| Total (159 days) | 20.693 | 23.637 | 11.550 | 13.955 |

The results are a reflection of the pathology of the scabby tubers and are an interesting addition to the physiology of abnormal growths. The loss of carbon dioxide was relatively heavy from the scabby

tubers during the early part of the season (October to February), but by February the scab lesions had healed so thoroughly that the skins of the scabby tubers were as impermeable as those of the clean ones. During March the scabby tubers should apparently have had an even better protection against the loss of carbon dioxide than the clean ones. The fact that the clean tubers produced more carbon dioxide late in the season was due to their earlier germination, as table 3 shows.

TABLE 3.—*Carbon dioxide production (milligrams per kilogram-hour) by clean and scabby potatoes during 3 periods in March, as determined by the absorption and sampling methods*

| Period | Absorption method | | Sampling method | |
|---|---|---|---|---|
| | Clean potatoes | Scabby potatoes | Clean potatoes | Scabby potatoes |
| Mar. 1 to Mar. 5 | 5.8 | 6.0 | 2.8 | 2.8 |
| Mar. 9 to Mar. 13 (sprouting in clean tubers begun) | 10.2 | 6.3 | 3.3 | 2.7 |
| Mar. 15 to Mar. 19 (sprouting in clean and some in scabby tubers advanced) | 11.7 | 7.8 | 6.4 | 5.6 |

Scab spots delay germination from 1 to 2 weeks, and during that time the carbon dioxide production is greater from clean tubers with their large sprouts.

### COMPARISON OF RESULTS OBTAINED BY THE TWO METHODS

As compared with the sampling method the absorption method gave a uniformly higher production of carbon dioxide from both clean and scabby tubers (table 4). The conditions under which the comparisons were made should be noted. When the absorption method is used all the air from the bell jar is swept out by the air current passing over the tubers; when the sampling method is used the air over the tubers remains the same and the carbon dioxide produced remains in it. However, the amount of carbon dioxide in the jars never approached 2 percent, the limit of determination by the Haldane apparatus.

TABLE 4.—*Daily carbon dioxide production (milligrams per kilogram-hour) by clean and scabby potatoes for the days after the tubers had been aired and then replaced under the bell jars, or other tubers substituted, as determined by the absorption and sampling methods*

| Date | Absorption method | | Sampling method | |
|---|---|---|---|---|
| | Clean potatoes | Scabby potatoes | Clean potatoes | Scabby potatoes |
| Dec. 9 | 2.9 | 6.1 | 2.0 | 4.2 |
| 10 | 3.6 | 6.0 | 1.5 | 1.9 |
| 11 | 5.1 | 6.1 | 1.5 | 2.2 |
| 12 | 3.4 | 6.0 | 1.4 | 2.0 |
| 13 | 3.3 | 5.9 | 1.3 | 1.8 |
| Jan. 7 | 3.7 | 5.3 | 3.1 | 3.5 |
| 8 | | | 1.8 | 2.0 |
| 9 | 3.9 | 5.0 | 1.8 | 1.8 |
| | | | 1.7 | 1.7 |
| 29 | 4.6 | 4.3 | 3.3 | 3.6 |
| 30 | | | 2.3 | 2.5 |
| 31 | 4.5 | 4.1 | 2.0 | 2.3 |
| Feb. 1 | | | 1.8 | 2.2 |

The amount obtained with the Haldane apparatus the first day after the tubers were placed under the bell jars was similar to that obtained by the other method, but the drop was abrupt after that time and the amount remained relatively small. The tubers used were stored in the same room as the bell jars and had been kept there for at least 1 week before they were used.

Every period after the changing of the tubers showed a similar slump in the rate of respiration as indicated by the Haldane apparatus. The carbon dioxide in the other bell jars must have had the tubers as its source. The internal, intercellular gases percolate out through the lenticels from the inside of the tubers. With this point in mind, a measurement of the internal gases of the tubers under each of the jars was made at intervals after a series of readings had been taken for carbon dioxide formation (tables 5 and 6).

TABLE 5.—*Carbon dioxide production (milligrams per kilogram-hour) by clean and scabby potatoes on days upon which the internal gases were also analyzed, as determined by the absorption and sampling methods*

| Date | Absorption method | | Sampling method | |
|---|---|---|---|---|
| | Clean potatoes | Scabby potatoes | Clean potatoes | Scabby potatoes |
| Nov. 5 | 5.8 | 7.8 | 4.3 | 8.1 |
| Nov. 27 | 2.3 | 4.1 | 1.3 | 1.5 |
| Dec. 18 | 3.4 | 6.3 | 1.3 | 2.0 |
| Jan. 17 | 4.0 | 5.1 | 2.1 | 2.2 |
| Feb. 16 | 4.4 | 4.2 | 2.3 | 2.4 |
| Mar. 4 | 6.1 | 6.6 | 1.2 | 1.0 |
| Mar. 21 | 11.0 | 8.2 | 8.6 | 8.4 |

TABLE 6.—*Percentage of carbon dioxide and of oxygen in the internal gases of stored potato tubers after the completion of the respiration tests*

| Date | After absorption method | | | | After sampling method | | | |
|---|---|---|---|---|---|---|---|---|
| | $CO_2$ | | $O_2$ | | $CO_2$ | | $O_2$ | |
| | Clean potatoes | Scabby potatoes | Clean potatoes | Scabby potatoes | Clean potatoes | Scabby potatoes | Clean potatoes | Scabby potatoes |
| Nov. 5 | 35.6 | 28.6 | 7.8 | 12.0 | 40.0 | 56.1 | 11.0 | 6.9 |
| Nov. 27 | 35.5 | 33.1 | 8.6 | 9.0 | 42.6 | 52.8 | 8.0 | 5.7 |
| Dec. 18 | 29.8 | 28.3 | 6.8 | 10.7 | 35.9 | 46.1 | 11.7 | 9.7 |
| Jan. 17 | 32.5 | 36.5 | 8.1 | 6.9 | 35.3 | 43.7 | 9.5 | 8.7 |
| Feb. 16 | 28.2 | 32.9 | 10.4 | 11.0 | 37.1 | 42.7 | 8.6 | 6.2 |
| Mar. 4 | 22.7 | 55.3 | 7.0 | 10.1 | 33.8 | 54.8 | 5.2 | 9.9 |
| Mar. 21 | 45.6 | 58.3 | 3.4 | 8.3 | 55.1 | 60.1 | 4.1 | 5.9 |
| Average | 32.84 | 39.00 | 7.44 | 9.71 | 39.97 | 50.90 | 8.30 | 7.57 |

When determinations were made by the sampling method the amount of carbon dioxide remaining in the internal gases of the tubers was higher in all except one instance (when it was about the same) than it was when the absorption method was used (table 6), indicating that in the passage of the air through the bell jar for 24 hours, a considerable percentage of the internal carbon dioxide found its way out of the tuber. Determinations by the absorption method

showed that during November and December, before the scab lesions had completely healed, the scabby tubers allowed more carbon dioxide to pass out than the clean ones, i.e., the percentage remaining within them was smaller. In January, however, and the months following, the percentage remaining inside the scabby tubers was higher than in the clean ones.

### AMOUNT OF CARBON DIOXIDE REQUIRED TO CHECK RESPIRATION

The total accumulated carbon dioxide under the bell jars used for sampling never approached 2 percent. Trials made to determine the percentage necessary to check respiration, not to prevent it entirely, indicated that 7 to 9 percent was sufficient and that the acidity of the juice changed from about pH 6.2 to pH 5.8. Such a high percentage of carbon dioxide could probably never develop in a storage bin. The 7 percent carbon dioxide slowed down respiration to a minimum, but at least 16 percent of carbon dioxide was required to prevent it entirely. Kidd (*4*) found that 20 percent would not only stop respiration but would also prevent sprouting.

### AMOUNT OF CARBON DIOXIDE ACCUMULATING AT BOTTOM OF BINS

In order to determine the quantity of carbon dioxide that would accumulate in a bin, potatoes were stored in two large open-topped cylinders, clean tubers in one and scabby tubers in the other. Before the potatoes were put into the cylinders, rods of barometer glass were so suspended that air could be withdrawn from depths of 1, 2, 3, 4, and 5 feet. When these experiments were started about March 15, 1931, the storage temperature was approximately 38° to 40° F. Samples were taken weekly, but the percentages of carbon dioxide remained the same as that of the surrounding air until April 15. By that time sprouting was common on both clean and scabby tubers, and the temperature rose to 50° to 52° and the percentage of carbon dioxide in the bottom of the cans rose to 0.7 percent. In 1933 the experiment was repeated, starting February 9 and continuing until May 3. The readings from clean and scabby potatoes were approximately the same, 0.4 percent at 1 foot, 1.3 percent at 2 feet, 1.8 percent at 3 feet, 1.7 percent at 4 feet, and 1.8 percent at 5 feet.

The percentages which appear in the later readings are higher than those of Smith (*8*), but Smith's data are from tubers stored in bins that were not necessarily airtight, whereas the data recorded in this work were from tubers stored in cans. The top layers even in a can of potatoes, however, interpose only a small obstacle to the outward diffusion of gases formed inside the can. If such a formation is slow, as occurs from potatoes stored at low temperatures, the diffusion may keep pace with the formation in a bin that is only 5 feet in depth. The holes between the tubers, as was found by actual measurement, represent 17.7 percent of the surface area of the large potatoes and 14.8 percent that of the small ones. According to Brown and Escombe (*2*), if 11.34 percent of a septum area is composed of holes, the diffusion is 60 percent that of an open vessel. The irregular shape of the holes undoubtedly added to their diffusion efficiency as compared with circular holes. The 15 to 18 percent area of the interstices between the tubers would accordingly mean a diffusion equal probably to at least 70 percent of that of an open vessel.

Other factors, such as shape of pores and depth to which they are embedded, velocity of wind stirring, and the percentage of any gas in the storage chamber, affect the rate of gas diffusion (*3*), but these factors were not investigated in the present work as the area of a tortuous passage from the bottom of a container filled with potatoes would be difficult to determine.

## DISCUSSION

Respiration was always less under the bell jars tested by the Haldane apparatus than in those through which the carbon-dioxide-free air was passed. This may be explained by the diffusion of gases through the pores of an impervious membrane. The skin of the potato tuber is the membrane broken by the openings which are the lenticels. The chamber containing the $CO_2$ is the potato.

Brown and Escombe (*2*) have shown that around each pore an area of gas collects; very dense at the pore, gradually becoming more diluted as the distance from the pore increases. This is their so-called "shell" of gas. Exactly the same shell of $CO_2$ must occur over each pore, i.e., over each lenticel of the tuber. Let us see how these shells of gas behave under the two treatments.

In the aspirator method, when air, free of $CO_2$, passes over a lenticel, at the entrance to which the percentage of $CO_2$ is approximately, let us say 28.6 percent, the shell of carbon dioxide is swept away, leaving a very sharp gradient between the internal gas of the tuber and the air rushing by the opening. The $CO_2$ is densest at the center of the tuber away from these pores. The $CO_2$ rushes out of the tuber, is swept along and measured as part of the respiration. This lowers the amount of $CO_2$ remaining inside the tuber, as shown in table 6. The apparent respiration $CO_2$ by this method therefore includes (1) the carbon dioxide in the jar; (2) the carbon dioxide surrounding the pores, i.e., the shells; and (3) part of the carbon dioxide which is normally within the tuber but which rushes out through the pores when air is swept over them for 24 hours.

In the sampling method, the air inside the bell jars is still. Obviously a larger shell of diluted $CO_2$ can be formed over the pore. The preparations are made for taking the sample. The air is stirred by a celluloid fan, breaking up the shells around the pores and distributing the $CO_2$ throughout the air of the bell jar so as to make a homogeneous gaseous mixture. The 10-cc sample is then taken immediately, the whole process involving only a few minutes. Some of the internal $CO_2$ rushes out through the pore, but the amount is negligible because the operation is of such short duration. The apparent respiration by the sampling method, therefore, includes $CO^2$ from only two sources: (1) that which is in the bell jar entirely free from the tubers; and (2) the shells of $CO_2$ distributed throughout the jar by the celluloid fan.

A comparison of tables 5 and 6 will show the correctness of the data on which the foregoing explanation is based. A discussion of the physical phase may be found in Brown and Escombe's paper (*2*) and the effect of currents of air in Huber's (*3*).

The aspirator method would most properly be used for studies on apple storage; the sampling method would be better for potatoes and root crops. Neither of these methods would give absolute correct-

ness. The same conclusion on potato storage may be drawn from this work that Smith (*8*) drew from his, namely, that carbon dioxide will never collect in sufficient quantity to make ventilation in a storage cellar necessary.

## SUMMARY

The respiration records of four lots of potatoes were studied through a storage period of 159 days. Two of these lots were composed of clean tubers and two of scabby tubers.

The respiration rate was obtained (1) on one lot each of clean and scabby tubers by passing carbon-dioxide-free air through the bell jars in which they were kept, the carbon dioxide being absorbed by an alkaline solution; and (2) by withdrawing 10-cc samples from one lot each of the clean and scabby tubers and determining the percentage of carbon dioxide by the use of a Haldane apparatus.

During the first month of the storage period the respiration rates of the scabby tubers were much higher than those of the clean tubers as the cork layers under the scab lesions were not so impervious to gases as the skin of the uninjured ones. The rates tended to become equalized during January and February, but after sprouting began in March the clean tubers respired more than the scabby ones, as sprouting was delayed for some time (7 to 10 days) by the scab lesions.

The respiration rates obtained by passing carbon-dioxide-free air through bell jars containing tubers were uniformly higher than those obtained by taking samples of the air in bell jars containing tubers. The reason suggested for the higher respiration rates obtained by the absorption method is that the flow of air through the jar removes the carbon dioxide not only from around the tubers but also from the gases held in the intercellular spaces. It is also suggested that the checking of respiration from tubers under the bell jars used in the Haldane apparatus tests may have been due to "shells" of carbon-dioxide-laden air around the opening of the lenticels. These shells are not disturbed in the air of the bell jar and check the outward diffusion of carbon dioxide.

The small amount of carbon dioxide which had accumulated inside the bell jars used for the Haldane apparatus tests was not sufficient to check respiration, 7 to 9 percent being required to check it.

The amount of carbon dioxide which appeared at the bottom of a special storage bin in which the tubers were 5 feet in depth was practically that of the surrounding air during the storage season and never rose until the tubers began to sprout, when as high as 1.8 percent occurred at the bottom with a temperature of about 50° F. This diffusion of carbon dioxide from the surface of potato bins follows the laws of the diffusion of gases through pores. With large tubers 17.7 percent of the surface was pore area, and this surface offered little resistance to the diffusion of the carbon dioxide formed at the bottom.

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